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Cause and Prevention of Liver Off-Flavor in Five Beef Chuck Muscles

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CAUSE AND PREVENTION OF LIVER OFF-FLAVOR IN FIVE BEEF CHUCK MUSCLES

by

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ABSTRACT

Cause and Prevention of Liver Off-flavor in Five Beef Chuck Muscles

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Liver off-flavor is a sporadic problem that limits the consumer acceptance of several beef chuck muscles, including the *infraspinatus* (flat iron steak). Residual blood hemoglobin is known to contribute to liver off-flavor development. This study was conducted to evaluate factors affecting development of liver off-flavor after cooking of beef chuck (shoulder) muscles. The study was conducted in three parts.

The objective of part 1 was to determine effects of muscle (*infraspinatus, longissimus dorsi, serratus ventralis, supraspinatus, teres major*) and processing (with or w/o carcass electrical stimulation) on residual blood hemoglobin content and total pigment content of raw muscle and sensory characteristics after cooking to 71 or 82˚C. The objective of part 2 was to evaluate the effect of antioxidant treatment and anaerobic packaging to possibly reduce the incidence of liver and other off-flavors of beef *infraspinatus* (IF) steaks. The objective of part 3 was to

determine the effect of animal age (commercial grade; >42 months, compared to select grade; <30 months), antioxidant treatment, and anaerobic packaging on sensory characteristics of beef IF steaks.

Among beef chuck muscles, the *infraspinatus* had highest mean liver flavor score of 2.08±1.00 where 2=slightly intense liver flavor. Other muscles (*longissimus dorsi*, s*erratus ventralis*, s*upraspinatus*, *teres major*) had mean liver flavor scores less than 2. Liver flavor score, myoglobin, hemoglobin, and total pigment content were higher ($p<0.05$) for *infraspinatus* muscle from older animals. Among select grade muscles, carcass electrical stimulation had no significant effect on liver flavor score. Rancid flavor scores were significantly increased from 1.34±0.65 to 1.58±0.84 as internal cook temperature increased from 71 to 82°C but mean TBA values as a measure of rancidity (0.25±0.15 and 0.29±0.13, respectively) were not affected by cook temperature.

Antioxidant treatment significantly reduced TBA values, rancid, and liver flavor scores for aerobically packaged steaks (PVC or 80% O2-MAP) but had little effect on scores of steaks in anaerobic packaging (0.4% CO-MAP). Results of this study indicate that *infraspinatus* steaks from older animals are most likely to have objectionable liver, sour/grassy, or rancid flavors. Objectionable flavor scores were lower in steaks receiving antioxidant injection or packaged anaerobically in 0.4% CO-MAP.

(124 pages)

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LIST OF SYMBOLS, NOTATION, DEFINITIONS

A – Absorbance

a* - Redness

b* - Yellowness

C – Celcius

CO – Carbon monoxide

DeoxyMb - Deoxymyoglobin

ES – Electrical stimulation

Hb – Hemoglobin

HPLC – High performance liquid chromatography

IF – *Infraspinatus*

LD – *Longissimus dorsi*

L* - Lightness

MAP – Modified atmospheric packaging

Mb – Myoglobin

MetMb - Metmyoglobin

MM – Milk mineral

O_2 – Oxygen

OxyMb - Oxymyoglobin

PVC – Polyvinyl chloride

PUFA – Polyunsaturated fatty acids

SERR – *Serratus ventralis*

SS – *Supraspinatus*

STP – Sodium tripolyphosphate

TBA – Thiobarbituric acid

TM – *Teres major*

WOF – Warmed over flavor

WBS – Warner Bratzler shear

CHAPTER I

INTRODUCTION

Meat quality is judged by its color and characteristic flavor. Liver off-flavor is a sporadic problem that limits the acceptance of beef chuck muscles. Stabilizing red color of beef and keeping it red for a longer period (more than 20 days at refrigeration) will increase beef sales. The other factor which affects beef consumption is its flavor. Consumers do not want to buy or consume beef that lacks beefiness or has off-flavors of any kind like rancid, metallic, sour/grassy and more importantly liver-like. Very little information is available regarding the cause of liver-like off-flavor in beef muscles. Studies were done by Lugay and Beale (1978) on beef muscles and they found that free ionic iron was associated with lipid oxidation and liver-like off-flavors in beef chuck muscles. Im and others (2004) found that residual blood was associated with liver off-flavor in beef muscles. *Infraspinatus* is a beef chuck muscle that is growing as a restaurant grade steak. It is also called flat iron steak and is found to be more prone to liver like off-flavor as compared to other four beef chuck muscles, *L. dorsi*; *teres major*; *supraspinatus*, and *serratus*.

Miller (2001) reported that higher levels of myoglobin, higher degree of doneness and higher amount of lipid oxidation enhanced metallic and liver-like off-flavors in beef cuts. In contrast Larick and Turner (1989) reported that phosphatidylethanolamine and lysophosphatidylcholine significantly contributed to the liver-like off-flavor in beef and bison steaks. However, there is no reference that strongly states the cause of liver off-flavor in particular. Liver-like

off-flavor is specific to individual animals and is found to be associated with other off-flavors such as rancid, oxidized, sour/grassy, or gamey (Meisinger et al. 2006).

The three sensory properties by which consumers judge meat quality are appearance, texture, and flavor. The most important of these is product visual appearance because it strongly influences the consumer's purchase decision (Kropf et al. 1986; Faustman and Cassens 1990). Acceptable fresh beef color is bright, cherry-red, and short-lived. Beef color is principally due to three pigments (Livingston and Brown 1981). Deoxymyoglobin (**DeoxyMb**) is the purple pigment observed in freshly cut meat. Following several min of exposure to air, DeoxyMb becomes oxygenated to oxymyoglobin (**OxyMb**), which has the characteristic bright, cherry-red color of beef. After several hours to several days of exposure to air, OxyMb gives way to metmyoglobin (**MetMb**) in which a molecule of water substitutes for a molecule of oxygen and results in brown pigmentation (Hui et al. 2001). Both DeoxyMb and OxyMb are heme proteins in which iron exists in the ferrous (Fe^{+2}) form, unlike MetMb, which possesses the ferric (Fe^{+3}) form. The conversion of the ferrous to ferric form is a result of oxidation. A diverse array of prooxidant molecules could initiate the oxidative process (e.g., ferric ions and reactive oxygen species such as superoxide anions, hydrogen peroxide, and hydroxyl radicals). These oxidizing species could interact directly with ferrous iron or cause the formation of lipid radicals and peroxides, which could oxidize the ferrous iron (Gutteridge and Halliwell 1990;

Minotti and Aust 1992; Schaich 1992). Lipid oxidation is a degradative process resulting in rancidity in uncooked meat and (or) warmed-over flavor (WOF) in cooked meat. It is one of the primary causes for deterioration of color, texture, and flavor of fresh, frozen, and cooked beef (Kanner 1994). Lipid oxidation is positively correlated with pigment oxidation (Hutchins et al. 1967; Greene 1969; Faustman et al. 1989), but the basis for this relationship is not understood. From the viewpoint of meat color, it may be that radical species produced during lipid oxidation act directly to promote pigment oxidation and (or) indirectly by damaging pigment-reducing systems. The storage of precooked meat for a short period results in the development of a characteristic off-flavor caused by catalytic peroxidation of phospholipids in biomembranes (Pearson et al. 1977; Kanner 1994). In addition to the consequence of lipid peroxidation on changes in meat flavor, color, and texture, the autoxidation of unsaturated lipids and cholesterol results in the generation of atherogenic compounds (Addis and Park 1989).

In this study liver off-flavor was observed to be associated with *infraspinatus* muscle of beef chuck and also sour/grassy off-flavor increased as the age of animal advances. The experiment was first designed to test the hypothesis that residual blood associated with incidence of liver off-flavor in five muscles of beef chuck of young animals but then it was found that flavor profile of muscles (especially *infraspinatus*) changes significantly with the age of animal. Thus, experiments were done to test the association of age with liver off-

flavor and sour/grassy flavors. Preventive measures were also examined to reduce the off-flavors mentioned above in both young and old animal *infraspinatus* muscles. It included a combination of antioxidants (0.3% sodium tripolyphosphate + 500 ppm ascorbic acid) and aerobic packaging (PVC-wrap and 80% O_2-modified atmospheric packaging (MAP)) and anaerobic packaging (0.4% Carbon monoxide-MAP). Tenderness was also tested both by trained sensory panel and instrumentally by Warner Bratzler Shear (WBS) force. Data were analyzed and observed for specific relationships between age and liver off-flavor incidence, age and sour/grassy flavor, electrical stimulation versus non electrical stimulation processing with liver off-flavor, effect of antioxidant and packaging methods on liver off-flavor (along with other off-flavors) and tenderness of *infraspinatus* muscle.

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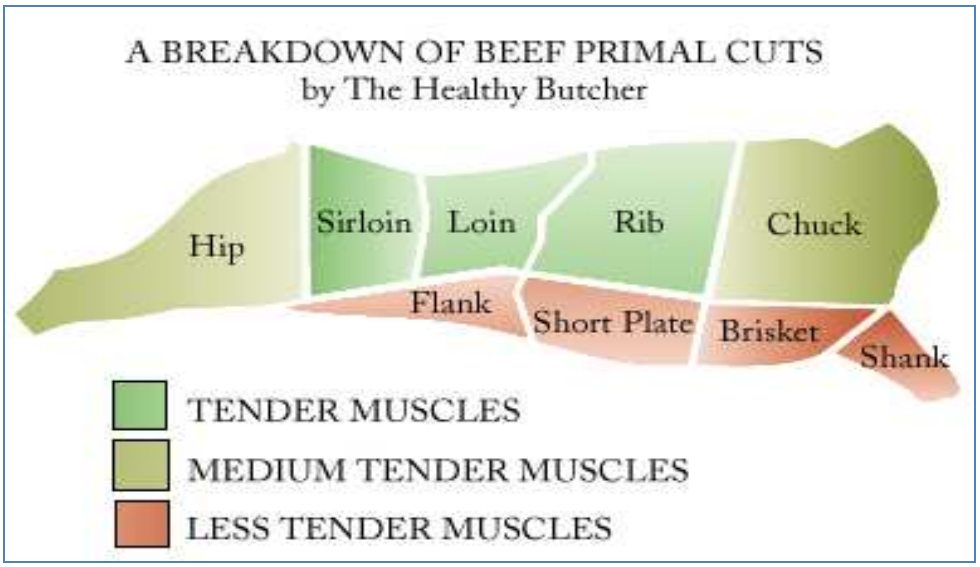
CHAPTER II

LITERATURE REVIEW

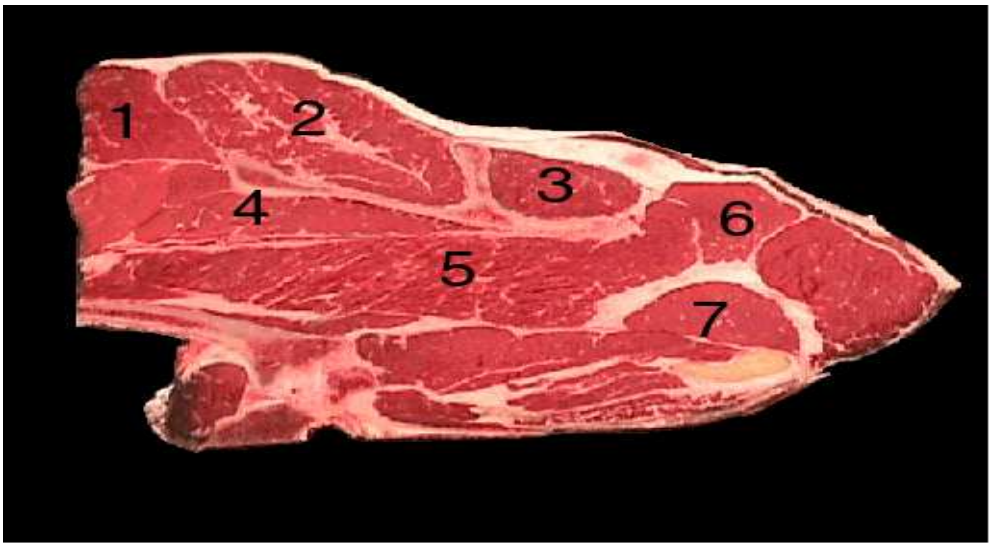
GENERAL INFORMATION ON BEEF CHUCK MUSCLES

Beef chuck or shoulder consists of around thirty muscles. *Infraspinatus, longissimus dorsi, terres major, serratus ventralis* and *supraspinatus* are five chuck muscles that were tested in this study. The anatomical location of these beef muscles is shown in the **Figure 1** and the chuck portion is separately shown in **Figure 2** and **Figure 3** with some chuck muscles localized together**.** Shoulder or chuck muscles are intermediate in their level of toughness and they are usually cooked for longer times using moist cookery methods in order to gelatinize connective tissues and make them tender. The anatomical location of some muscles has been discussed by Swatland (1984). The *supraspinatus* is dorsal to the spine or ridge on the scapula, while the *infraspinatus* is ventral to the scapular spine. The *trapezius* is located superficially between the left and right scapular blades. The *rhomboideus* is ventral to the *trapezius*. The *subscapularis* is located on the flat medial face of the scapula, towards the ribs. The *biceps brachii* is anterior to the humerus in an equivalent position to the biceps muscle in the human arm. The *triceps brachii* is a large muscle located in the triangular area bounded by the ventral edge of the scapula and the posterior edge of the humerus. So the definition of five beef chuck muscles used experimentally in

this study (Swatland 1984) is given below (based on their anatomical location):

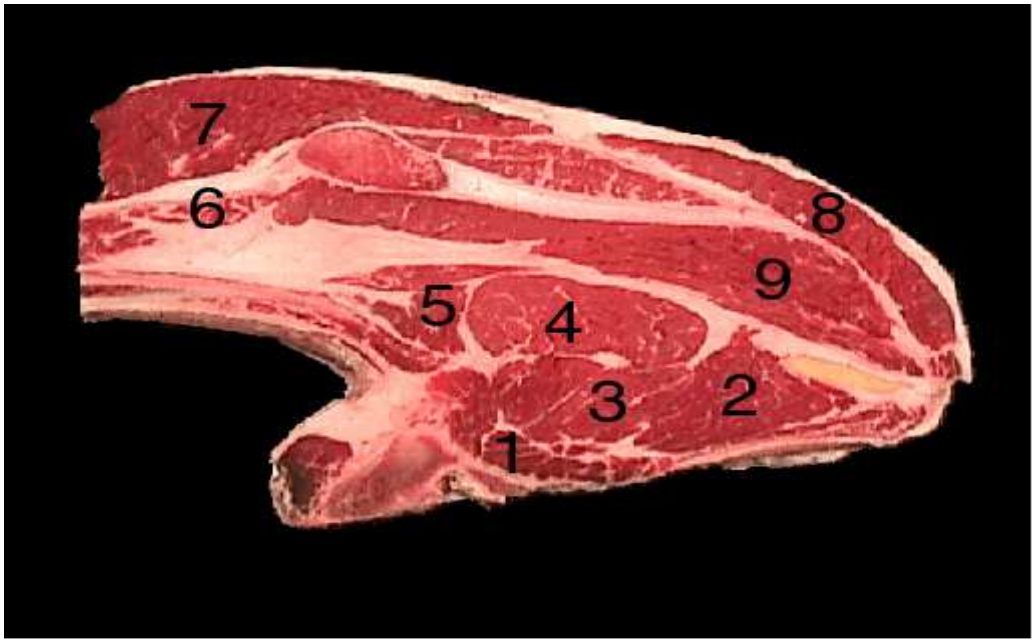


(www.thehealthybutcher.com/livetoeat/volume2/B)
Figure 1 Beef chuck (shoulder) muscle anatomical location



www.meat.tamu.edu/anatomy/images/7bone.jpeg
Figure 2 Beef chuck muscles localization within the chuck; cross-section at the 2nd rib

1. Triceps brachii; 2. *Infraspinatus;* 3. *Supraspinatus;* 4. *Subscapularis;* 5. *Serratus ventralis;* 6. *Rhomboideus;* 7. *Complexus*



www.meat.tamu.edu/anatomy/images/blade.jpeg

Figure 3 Beef chuck muscles localization within the chcuk (continued); cross-section at the 5th rib

1. *Multifidus dorsi;* 2. *Spinalis dorsi;* 3. *Complexus;* 4. *Longissimus dorsi;* 5. *Longissimus costarum;* 6. *Serratus ventralis;* 7. *Latissimus dorsi;* 8. *Trapezius;* 9. *Rhomboideus*

Infraspinatus – A large muscle almost filling the *infraspinatus fossa* ventral to the scapular spine.

Teres major – A muscle attached to the posterior corner and adjacent posterior border of the scapula.

Longissimus dorsi – Its full name is *longissimus thoracis et lumborum*. It is a long muscle that forms the large round eye of meat in rib and loin steaks or chops. It is also defined as a muscle lying ventrally to the transverse processes of the vertebrae.

Serratus ventralis – The *serratus ventralis* is a large fanlike muscle that radiates out from the medial surface of the scapula. It is composed of easily visible radiating subunits which end ventrally in a sawtooth pattern, hence the muscle's name. It can be divided as *serratus ventralis* thoracis and *serratus ventralis* cervices.

Supraspinatus – A large muscle filling the *supraspinatus fossa* dorsal to the scapular spine.

OFF-FLAVORS ASSOCIATED WITH BEEF

Meat quality is often determined by color, flavor and tenderness (Cornforth 1994). Flavor is the one very likely tool to judge the liking and disliking of meat. More than one thousand volatile compounds have been identified in beef or meat (Moss and Calkins 2007a). Consumers need training to identify off-flavors so that trained panelists always give reproducible results as compared to untrained panelists. Some of the off-flavors commonly associated with beef are rancid, oxidized, metallic, sour, fatty, burnt, salty, bloody, grassy, livery, painty, or gamey (Moss and Calkins 2007a).

Rancid flavor and TBA scores were studied by Gokalp et al. (1983) as influenced by packaging. TBA scores were the measurement of malonaldialdehyde which forms due to oxidation of lipid molecule. The score

above 1 is considered rancid. They found that in the neutral lipid fractions, oxidative degradation of the C16:1 and C18:2 fatty acids made significant contributions to increased rancidity scores and TBA values but changes in the C18:3 and C20:4 were of greatest importance in the phospholipid fraction. They also found that after 3 months of frozen storage, fatty acids C18:1 and C18:2 and the phospholipid fatty acids C18:3 and C20:4 accounted for 88% and 85% of the variation in rancidity score and TBA value. Many researchers worked to determine the causes and preventive methods for all off-flavors but very little information is available for liver-like off-flavor.

FACTORS CONTRIBUTING TO THE OFF-FLAVORS IN BEEF

Meisinger et al. (2006) found that there is an "animal effect" to off-flavors found in the chuck and knuckle, meaning the animal has been exposed to something different than a normal animal. The advent of many off-flavors in meat is found to be associated with lipid oxidation so methods used for determining lipid oxidation like TBA (thiobarbituric acid) test, descriptive panel for sensory evaluation of beef flavors like rancid, metallic, beefy and livery flavors have been evaluated. Flavor is a very complex attribute of meat palatability. The beef customer satisfaction survey found many factors that influenced overall like ratings of top round, top sirloin, and top loin steaks with flavor and tenderness contributing equally to overall like ratings (Lorenzen et al. 1999; Neely et al. 1998, 1999; Savell et al. 1999). Calkins

and Hodgen (2007) stated that complex interactions between various compounds influence the perception of meat flavor. Inherent flavor of a meat product can be influenced by oxidation, lipid content, feeding/diet, myoglobin, and pH.

Effect of animal age on off-flavor incidence

It has been observed that beef from mature cow is tougher than from young animals. Dunsing (1959) investigated consumer preferences of beef varying in chronological age and determined that consumers consistently prefer beef from younger animals because of increased tenderness. Injection of 0.3 M $CaCl_2$ (10% of subprimal weight) improves tenderness of beef from older animal if injected within 30 min of slaughtering (Morgan et al. 1991).

Aberle and others (1975) reported that animal aging was accompanied by darkening of muscle color due to increased myoglobin concentration. Dark color of muscle is useful only as a guide of animal age. They also discussed that substantial muscle thickening in beef animals is evident at about 30 months of age. Meat flavor intensity also increases with animal age. The likely cause of this flavor change is increased concentration of nucleotides in muscle, which degrade to inosinic acid and hypoxanthine postmortem. Flavor intensity may become so great that it is objectionable to some consumers, an example being the strong mutton flavor of mature sheep or game animals. However, any change in the flavor or color largely depends on animal species, breed, and other anatomical and nutritional factors. Another study done by

Shorthorse and Harris (1990) showed that *psoas major* muscles were unaffected by increasing animal age whereas high connective tissue strength muscles, such as the *biceps femoris*, trebled in toughness.

Effect of animal diet

Diet plays an important role in characterizing muscle flavors in both ruminants and nonruminants. Any feeding practice in the immediate antemortem period which alters the quantity of glycogen stored in muscles can influence the ultimate physical properties of meat. Feeding animals higher levels of vitamin results in improved product quality. One in particular, is Vitamin E. Vitamin E is fat soluble and therefore, is deposited in lipid rich tissues and cell membranes. Vitamin E is a powerful antioxidant and likely improves the shelf life and color of fresh meat by inhibiting fat and myoglobin oxidation. High forage diets produce some undesirable meat flavor compounds. A variety of unusual or 'grassy' flavors have been noted (Aberle et al. 1975) which are caused by a variety of compounds in forages. The problem may be eliminated by feeding grain diets for several weeks before slaughter. A special flavor problem sometimes exists in meat of pastured animals that consume wild onion or wild garlic (Aberle et al. 1975).

New research reveals important relationships in flavor among multiple muscles within a single animal carcass. This animal effect includes the presence of off-flavors. Diets high in polyunsaturated fatty acids may be contributing to the appearance of off-flavors in beef including fishy flavors (Poulson et al.

2004). Compounds associated with liver-like off-flavor notes in beef have been identified in raw tissues. Wood and Enser (1997) showed that oxidation could be controlled by the amount of antioxidant compounds found in the muscle tissue. Grass-fed beef may be less prone to lipid oxidation than grain-fed beef because of the increased levels of vitamins A, C, and E, cartonenoids, and flavonoids found in forages. It has been stated by Moloney and others (2000) that beef tenderness, juiciness and flavor can be altered by feeding cows with special diets. Flavor of beef is influenced by diet, but assessment of flavor by a consumer taste panel is subject to the previous experiences and preferences of the panelists.

Modern lean beef can have an intramuscular fat concentration of 25–50 g/kg and can be considered a low-fat food. As the quantity of grass in the diet of cattle is increased, there is a decrease in saturated fatty acid concentration and an increase in the n-3 polyunsaturated fatty acid and conjugated linoleic acid concentrations. It is concluded that there is opportunity to exploit the diet of cattle to produce tender flavorful beef that has an increased conjugated linoleic acid concentration, a lower fat concentration and a fatty acid profile more compatible with current human dietary recommendations. Moss and Calkins (2007b) reported that feeding a high energy diet for at least 60 days prior to harvest changes the flavor of cow beef. Moreover, Mandell and others (1998) concluded that palatability attributes of ribeye roasts and ground beef were generally unaffected by diet with the exception of slightly less beef flavor and more off-flavor in forage-fed than in grain-fed beef.

Effect of cooking medium and heating

The effects of belt grill and open hearth electric broiler cookery on palatability and cooking traits of *longissimus* steaks were done by Wheeler and others (1994). The *longissimus thoracis* from carcasses of grain-fed steers or heifers was used. They found that belt grilled steaks had lower cooking losses (20.2 vs 29.8%); higher tenderness (7.0 vs 6.7) and juiciness (6.0 vs 5.1); and lower connective tissue amount (7.7 vs 7.8), beef flavor intensity (5.0 vs 5.1), and off-flavor (3.2 vs 3.3) ratings than steaks cooked with the electric broiler. Another study done by Schock and others (1970) found that the rate of heat penetration, cooking time, cooking losses, total moisture, press fluid, water-holding capacity and juiciness varied and apparent degree of doneness varied among the 4 heat treatments (deep-fat fried, oven-roasted, oven-braised, and pressure-braised at 10 psig to an internal temperature of 70°C). They also found that tenderness and flavor influenced overall acceptability scores more than juiciness or apparent degree of doneness. A review article has been published in 2006 (King and Whyte 2006) mentioning the factors that influenced the color of cooked meat. Consumers are more likely to assess cooking status by the color of the meat or juice. This article reviewed the factors that can influence the final color of cooked meat. In most instances, these factors influence color by modifying the meat pigment myoglobin prior to and during cooking. Many factors can prolong the pink

"uncooked" color in meat, including high pH, modified atmosphere packaging, rapid thawing, low fat content, nitrite, and irradiation. Such factors may lead to overcooking and loss of food quality, and consumer rejection. Alternatively, factors that cause "premature browning" of meat, where the interior of the product looks cooked but a microbiologically safe temperature has not been reached, are food safety issues. Pale, soft and exudative meats can prematurely brown, as can meats packaged under oxygenated conditions, frozen in bulk or thawed over long periods, or those that have had salts or lean finely textured beef added. Meats cooked from a frozen state or irradiated in aerobic conditions might also be at risk, but this might depend on meat species. In summary, the color of cooked meat is not a good indicator of adequate cooking, and the use of a food thermometer is recommended and internal temperature of cooked meat should be not less than 71°C for food safety purposes.

The acceptability of meat to consumers is largely determined by texture, flavor and color of the product after commercial or home cooking (Byrem and Strausburg 2000). While each of these characteristics may be influenced to some extent by antemortem factors, such as breed, species, diet, and nutritional status, postmortem factors such as biochemical status of the product, cookery method, cooking time, end point temperature and fat levels also influence acceptability of meat. Cooking of meat results in production of a complex variety of hundreds of flavor compounds. This again depends on

animals' species, breed, diet and fatty acid composition. Heat treatment also results in color changes in meat. Slow cooking of a red meat at 60°C may yield product with significant retention of redness in red meat. Whereas fast cooking at higher temperature may yield a product with browning reactions.

LIVER FLAVOR IN MEAT – SERIOUS CONSUMER PROBLEM

Due to increased number of complaints for liver off-flavor in meat (which is a sporadic but serious problem), there has been adverse effect on the sale of beef muscles from the chuck. Traditionally, muscles of the beef chuck have been marketed as bone-in blade and arm roasts or ground beef. However, recent work has identified several muscles in the beef chuck that are sufficiently large and tender that they can be removed and marketed as individual boneless steaks, allowing processors to capture the greater market value of steaks, compared to roasts or ground beef (Seggern et al. 2005). Egan and Shay (1982) characterized liver like flavor as developed in vacuum packaged meat stored at 5˚C in absence of microorganisms.

The *infraspinatus* or flat iron muscle is one example of a shoulder steak that has received widespread market acceptability in recent years. However, several of the chuck steaks, including the *infraspinatus*, have a higher than normal incidence of liver or metallic flavor after cooking. Even though liver off-flavor is sporadic, it nonetheless limits demand for beef, since consumers who have an undesirable experience are less likely to purchase the

same or similar products in the future. Thus there is a need to better understand factors associated with liver flavor development, so that preventative measures may be implemented.

CAUSES OF LIVER FLAVOR

Very little information is available on causes of liver flavor in meat products. It was hypothesized that since foodservice preparation traditionally cooked the meat quickly and then held the product in warming ovens until the food was presented to the consumer these conditions might promote the liver-like flavor (Calkins and Hodgen 2007). However, it has been conclusively shown that off-flavor in porcine liver is associated with residual blood (Im et al. 2004). These authors noted that when blood was completely removed by insertion of a cannule into the major vein and running 0.9% NaCl solution through the liver, no fishy or metallic off-flavors were found. These authors further noted that even very low levels of ferrous chloride exposure to porcine liver caused off-flavor development. Using headspace solid phase micro-extraction to trap volatiles, followed by gas chromatography, Im and others (2004) demonstrated that lipid oxidation products were associated with liver off-flavor, as follows: 1-octen-3-one (metallic), hexanol (weak metallic), and 2-nonenal (cardboard-like). Oxidation of arachidonic acid was associated with metallic and liver flavors, while oxidation of linolenic acid was associated with fishy flavors (Im et al. 2004). Lugay and Beale (1978) also

demonstrated that blood is associated with liver flavor. They developed a textured pet food with the flavor and consistency of liver by heating a homogenized mixture of fat, water, blood and a reducing sugar. Werkhoff and others (1996) worked to find the aroma concentrate from cooked beef liver and found that twenty-one organoleptically interesting mercapto/methylthio-substituted aldehydes and ketones identified in cooked beef liver for the first time including seven sulfur compounds which had not been previously described.

Myoglobin, with a molecular weight of 16,500 contains 1 heme group and one iron per molecule, while hemoglobin (MW = 64,500) contains 4 heme groups (four iron atoms) per molecule (Oellingrath and others 1990). Seggern and others (2005) measured total heme concentration of various beef muscles as an indication of total muscle pigment content. Among chuck muscles, they found that the *biceps brachii* and the *multifidus /spinalis dorsi* had highest values of 24.7 and 24.9 ppm heme iron, respectively. The hemoglobin content of a muscle depends upon the extent of the vascular bed in the muscle and the bleeding of the carcass (Oellingrath et al. 1990). Generally only about 10% of the total pigment of meat is hemoglobin (Warriss and Rhodes, 1977). While total heme content is an indication of myoglobin + hemoglobin content, the actual hemoglobin content must be measured directly. Oellingrath and others (1990) have reported a rapid high-pressure liquid chromatography method for measurement of both myoglobin

and hemoglobin in muscle homogenates, using a hydrophobic interaction column, which was found to be superior in separation and pigment recovery, compared to gel filtration or ion exchange columns.

Effect of animal diet on liver flavor

Vasta and others (2007) have discussed in their review article that feed or diet of the animal affected overall meat quality and flavor in particular. For instance, tannins can positively influence meat color and quality and protein content, but the effects depend on tannin concentration. In their article, they clearly mentioned that grazing saltbush (Atriplex spp.) preserves lamb meat color stability, suggesting that the high level of Vitamin E in these shrubs protects myoglobin from oxidation. Also they have investigated meat muscular fatty acid composition in lambs fed carob pulp with or without polyethylene glycol (PEG) supplementation, or maize. The intramuscular fat of lambs offered the carob based diet contained slightly higher levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), and lower levels of polyunsaturated fatty acids (PUFA) and n-6 fatty acids compared to lambs fed the maize-based diet.

Poulson and others (2004) mentioned that raising cattle on forage and pasture with no grain supplementation enhances beef conjugated linoleic acid (CLA) content. Additionally, finishing cattle on pastures increased the Vitamin E of beef by 300% compared to beef from animals finished on a

traditional high grain diet. The nutritional quality of meat from forage-fed animals is healthier than that from grain-fed animals. The meat of animals fattened on forages contains more β-carotene, Vitamin A, Vitamin E, omega-3 fatty acids, a healthier ratio of omega-3:omega-6, and more conjugated linoleic acid (CLA), all substances with favorable effects on human health (Dhiman et al. 2005).

Role of hemoglobin iron in liver off-flavor development

Blood likely contributes two precursors to liver flavor development; iron and polyunsaturated fatty acids (PUFA). As discussed above, the heme groups of hemoglobin contain iron, while the red blood cell membranes contain arachidonic acid and linolenic acid, which would serve as substrates for lipid oxidation in the presence of oxygen. Red blood cells may also contribute an essential third component for lipid oxidation, namely oxygen, since their well-known function is oxygen transport to the tissues.

As demonstrated by Im and others (2004) and Lugay and Beale (1978), free ionic iron greatly accelerates lipid oxidation and liver off-flavor development. Iron in raw beef muscles exists primarily as a component of the heme group of myoglobin and hemoglobin. However, heating (cooking) causes heme degradation, with an increase in ionic iron content proportional to the severity of heating (Buchowski et al. 1988). Thus, it is postulated that

liver off-flavor may intensify with increasing internal cook temperature, as more free iron becomes available to catalyze lipid oxidation.

Free ionic iron plays two roles in lipid oxidation. First, iron ions catalyze the well-known decomposition of hydrogen peroxide (H_2O_2) to dioxygen (O_2), hydroxyl ion (OH^-) and hydroxyl radical ($OH\bullet$) as follows (Haber and Weiss 1934):

$$Fe^{+3} + O_2^- \rightarrow Fe^{+2} + O_2$$

$$\underline{Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH\bullet + OH^-}$$

$$O_2^- + H_2O_2 \rightarrow O_2 + OH\bullet + OH^-$$

Hydroxyl radicals are very reactive, capable of rapid removal of hydrogen atoms ($H\bullet$) from nearby lipids to form reactive alkyl radicals ($R\bullet$), in the initiation step of lipid oxidation (Brown and Fridovich 1980). Secondly, iron also catalyzes decomposition of lipid hydroperoxides (ROOH) during the propagation step of lipid oxidation, as follows (Nawar 1996):

$$ROOH + Fe^{+3} \rightarrow Fe^{+2} + ROO\bullet + H^+$$

$$\underline{ROOH + Fe^{+2} \rightarrow Fe^{+3} + RO\bullet + OH^-}$$

$$2ROOH \rightarrow RO\bullet + ROO\bullet + H_2O$$

Effect of animal age on liver flavor

Roth and others (1995) reported that there was limited effect of animal age on flavor notes of chevaline longissimus muscle including liver flavor. No significant information was found as association of animal age with liver flavor in particular. But Meisinger and others (2006) found that liver off-flavor is specific to individual animal and also associated with other off-flavors such as metallic, rancid, sour/grassy, and/or gamey. Higher myoglobin levels in bull meat have been suggested to lead to greater sensations of metallic, liver, serumy/bloody, and bitter flavors (Miller 2001). Beef from bulls was found to have higher livery odor and flavor and bloody flavor than heifers which were found to be related to higher 2-propanone levels using multiple regression and discriminant analysis (Gorraiz et al. 2002).

PREVENTION OF LIVER OFF-FLAVOR

It is the hypothesis of this study that residual blood hemoglobin and total pigment (myoglobin + hemoglobin) cause liver flavor in beef chuck muscles. So, complete bleeding is the most obvious way to avoid the problem. Postmortem electrical stimulation (ES) increases blood loss from the carcass, compared to non-stimulated carcasses (Smith 1985). Currently in the USA, most plants use low voltage ES in part to obtain the benefit of more complete blood removal from the carcass, and thus less blood drip in the

coolers. However, there may be animal-to-animal and even plant-to-plant differences in blood removal, in part due to differing plant procedures for ES.

Electrical stimulation of freshly slaughtered carcasses has been used successfully to improve tenderness and meat quality in turkey, lamb, beef, and veal. However, ES in pork is generally unacceptable because ES hastens postmortem metabolism to a point where it is detrimental to ultimate product quality. Tenderness improvement occurs in almost all carcasses subjected to ES, but the extent of improvement depends on inherent tenderness of carcasses. ES is most appropriate for carcasses of young animals that have not been fed high energy diets or that lack inherent tenderness.

Chuck muscles are often high in red; type 1 muscle fibers, which are associated with greater capillary volume than white fibers (Hedrick et al. 1994). It may not be possible to remove all capillary blood from some beef chuck muscles. So, alternative preventative measures are needed. Since liver off-flavor development is caused by lipid oxidation, liver flavor development can be inhibited by 1) oxygen removal, or 2) addition of antioxidants. Lipid oxidation and rancidity can be greatly slowed by packaging beef steaks or ground beef in vacuum or in low oxygen, modified atmosphere packaging with 0.4% carbon monoxide (John et al. 2004, 2005). For fresh beef steaks, CO-MAP packaging will maintain products in a bright red state, while vacuum packaged products will appear dark purplish red. High (80%) oxygen packaging is also widely used to maintain bright red appearance of fresh beef

steaks, but rancid flavors become detectable by 6 days storage (Jayasingh et al. 2002). Therefore, there is great interest in antioxidants for use with fresh meats packaged in a high oxygen environment. Antioxidants may be classified as either type 1, such as vitamin E, or type 2, such as sodium tripolyphosphate (STP). Type 1 antioxidants are capable of donating a hydrogen atom (H•) to reactive free radicals, thereby slowing the propagation step of lipid oxidation (Brown and Fridovich 1980). Type 2 antioxidants bind ionic iron, removing iron as a catalyst for initiation and propagation steps of lipid oxidation. Polyphosphates such as STP, phytic acid, and milk mineral (MM) are very effective at binding iron and slowing lipid oxidation in cooked ground meats (Lee et al. 1998; Cornforth and West 2002). STP and especially milk mineral (MM) were recently found to greatly slow both lipid and myoglobin oxidation in fresh ground beef in 80% oxygen MAP (Vissa and Cornforth 2006). MM is a fine white powder. It consists of insoluble calcium phosphate particles, derived by drying whey permeate after the ultrafiltration removal of lactose and protein. While MM is an effective antioxidant in ground meats (Cornforth and West 2002), STP is more soluble in water or brines, and would have greater applicability for injection into steaks or roasts. Injection of fresh beefsteaks and roasts with solutions of salt and STP is now permitted by the USDA. Thus, "enhancement" injection of fresh beef is increasingly used, particularly in MAP products. Injection of beef loin strips with phosphate, lactate, and sodium chloride improved beef tenderness and

juiciness (Vote et al. 2000). Mancini and others (2005) used enhancement solutions of lactate and rosemary to improve strip loin steak color stability, and reported that lactate-enhanced products were darker but had a more stable red color.

To prevent or reduce off-flavors associated with lipid oxidation including rancidity and liver off-flavor and to prevent browning, it is permitted to dip or spray fresh meats with antioxidants solution containing water soluble antioxidants (0.3% STP + 500 ppm sodium ascorbate) (Cheng 1987; Manu-Tawiah et al. 1991).

PREVENTION OF OTHER OFF-FLAVORS IN BEEF

In addition to liver off-flavor prevention in beef, studies have been done to prevent the incidences of other off-flavors, especially warmed over flavor (WOF), rancid, metallic, in beef. Angelo and others (1990) stated that free radical scavengers appeared overall the most effective inhibitors of WOF. Rojas and Brewer (2007) reported that grape seed extract at 0.02% had the potential to reduce oxidative rancidity and improve shelf life of refrigerated cooked beef and pork patties. Park and others (2007) evaluated lipid oxidation and oxidative volatiles as affected by pork meat cut and packaging method during frozen storage at –10 °C and concluded that peroxide values increased with increased storage time, but were not affected by pork meat cut and packaging method.

They also reported that most of the volatiles decreased with prolonged storage except propane.

The rosemary extracts showed significant protection of lipid oxidation and color change in cooked turkey. Higher levels of water-soluble rosemary extracts were more effective in delaying quality loss in cooked turkey at all tested storage times (Yu et al. 2002). Vasavada and Cornforth (2006) examined the effect of raisin paste added to ground beef as an antioxidant. They reported that highest antioxidant effects were obtained with a minimum of 1.5%, 2.0%, or 2.0% raisin paste in cooked ground beef, pork, or chicken, respectively. There was a high correlation (0.93, 0.94, and 0.94) between TBA values and sensory rancid flavor scores in beef, pork, and chicken samples respectively. Chen and others (1999) noted that rosemary oleoresin and rutin were effective only in irradiated raw pork for 3 days. They also reported that hexanal, propanal and higher boiling components were well correlated ($p < 0.01$) with TBA in cooked pork and generation of volatiles was reduced by sesamol and quercetin, but the effects of antioxidants on color changes of raw pork patties were minor and inconsistent. Vissa and Cornforth (2006) observed the effect of milk mineral (MM), sodium tripolyphosphate (STP) and vitamin E (E) as potential antioxidants with 80% oxygen modified atmospheric packaging (MAP). They reported that thiobarbituric acid (TBA) values were highest in controls without antioxidants, and samples with added vitamin E. They explained that the lipid soluble vitamin E probably did not

mix thoroughly with ground beef, and thus had no antioxidant effect. Lowest TBA values were in samples treated with 0.75% or 1.5% milk mineral. All ground beef samples maintained redness through 4 d of storage and by day 7, ground beef treatments with added vitamin E were brown. After 14 d, ground beef with 0.75% MM was redder than other treatments or controls. They concluded that 0.75% MM had possible application to prolong red color stability and inhibit lipid oxidation in ground beef. Ascorbic acid was also found to inhibit lipid oxidation when added to beef at 0.2% (Srinivasan et al. 1996) but in contrast ascorbic acid is also able to serve as a donor antioxidant in free radical mediated oxidative processes, thereby increasing the pro-oxidant chemistry of the metals present (Buettner and Jurkiewicz 1996).

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CHAPTER III

HYPOTHESES AND OBJECTIVES

The hypotheses of this study is that residual blood hemoglobin or higher levels of total pigment (myoglobin and hemoglobin) causes liver off-flavor in beef chuck muscles, especially in older, commercial grade animals since it is known that off-flavors also are more prevalent in muscles from older animals. Therefore, to test the hypotheses, the objectives of the study were in three parts.

The objective of part 1 was to determine effects of muscle (*infraspinatus, l. dorsi, serratus ventralis, supraspinatus, teres major*) and processing (with or w/o carcass electrical stimulation) on residual blood hemoglobin content and total pigment content of raw muscle and sensory characteristics after cooking to 71 or 82˚C.

The objective of part 2 was to evaluate the effect of antioxidant treatment and anaerobic packaging to possibly reduce the incidence of liver and other off-flavors of beef IF steaks.

The objective of part 3 was to determine the effect of animal age (commercial grade; >42 months compared to select grade; <30 months), antioxidant treatment and anaerobic packaging on sensory characteristics of beef IF steaks.

CHAPTER IV

EFFECT OF MUSCLE TYPE AND PROCESSING CONDITIONS ON INCIDENCE OF LIVER OFF-FLAVOR IN FIVE BEEF CHUCK MUSCLES

INTRODUCTION

Liver off-flavor is a sporadic problem that may limit acceptance of beef chuck muscles including the *infraspinatus* (flat-iron steak). Residual blood hemoglobin is hypothesized to be the cause for liver-like flavor in beef muscles, specifically the heme iron present as a prosthetic group in hemoglobin. Little information is available on cause and prevention of liver flavor in meat. Im and others (2004) concluded that off-flavor in porcine liver was associated with residual blood. Lugay and Beale (1978) demonstrated a relationship between blood and liver flavor. They developed and patented a textured pet food with the flavor and consistency of liver by heating a homogenized mixture of fat, water, blood, and a reducing sugar.

Previous work has demonstrated that cooking causes heme degradation with an increase in ionic iron content (Buchowski et al. 1988). As demonstrated by Im and others (2004) and Lugay and Beale (1978), free ionic iron accelerates lipid oxidation (rancid flavor) and liver off-flavor development. Miller (2001) reported that beef cuts with higher levels of myoglobin, higher degree of doneness or with higher degree of lipid oxidation typically express a liver-like or metallic flavor.

Yancey et al. (2006) identified 13 compounds that were higher in samples with liver-like flavor when compared to samples without liver like off-flavors. Of these by products, six were aldehydes formed from oxidation of oleic and linoleic acid. Jenschke et al. (2007) reported that liver like and metallic off-flavors were more frequent in Choice than in Select carcasses. As lipid oxidation progressed, other off-flavors also increased.

In contrast, Yancey et al. (2006) concluded that liver flavor was not related to lipid oxidation but instead was a complex trait that could not be related to any single characteristic. Gorraiz et al. (2002) reported that bull beef had stronger liver flavor and odor that was related to a higher 2-propanone content and lysophosphatidyl choline. They also reported that aging of meat increased characteristic beef flavor and aftertaste.

It is the hypothesis of this study that that residual blood hemoglobin or higher levels of total pigment (myoglobin and hemoglobin) causes liver off-flavor in beef chuck muscles. Thus complete bleeding is the most obvious way to avoid the problem. Postmortem electrical stimulation (ES) increases blood loss from the carcass compared to non-stimulated carcasses (Smith 1985). The objective of this study was to determine the incidence of liver flavor among 5 beef chuck muscles as affected by muscle type, processing method (with or w/o electrical stimulation), and cooking temperature.

MATERIALS AND METHODS

Color Measurement

The color was measured on all five beef chuck muscles before cooking by using the Hunter Lab Miniscan portable colorimeter with a 5 mm aperture (Hunter Associates Laboratories, Inc., Reston, VA). The instrument was standardized using white and black standard plates and then CIE L*, a* and b* values were measured using illuminant D65. The a* value represents redness so brighter red the samples, higher the a* values.

HPLC

Sample preparation: Lean beef muscle (1000 g) was ground through a fine plate (3 mm diameter pore size) of a Hobart grinder (Model model 4152; Hobart Mfg. Co., Troy, OH). Ground beef samples (10 g) were diluted with 30 mL of 0.1 M sodium phosphate buffer pH 7.0 in a stomaching bag, and mixed for 1 min. at high speed using a Colworth Stomacher® 400 Model BA7021 (London, UK). The entire mixture was transferred to a 50 mL polypropylene tube and centrifuged (Sorvall Instruments, Model RC 5C, Dupont, Wilmington, DE) at 21,500g for 30 min at 2-5˚C. The supernatant was then filtered through a 0.45-micron membrane filter disc (Millipore, Billerica, MA). A 2 mL portion of filtered solution was diluted to 10 mL with 0.1 M sodium phosphate buffer. A few grains of potassium cyanide were added to maintain the heme pigments in their cyano-ferric form. Filtered samples (40 µl) were transferred to a 100 µl plastic, tappered bottom vial. The vial was placed on the holding rack of the

HPLC autosampler. The autosampler was set to withdraw 25 µl from each sample vial for pigment separation and quantitation.

Instrument description: A Beckman System Gold HPLC unit (Beckman Instruments, Inc., Fullerton, CA) with Autosampler 507 and UV detector instrument was used to run extracted beef samples for determining hemoglobin and myoglobin content. A hydrophobic column (Bio-Gel TSK Phenyl-PW 7.5 cm x 7.5 mm; Tosoh Biosciences LLC, Montgomeryville, PA) was used. The column was operated with 1.7 M ammonium sulphate, 0.1 M sodium phosphate as eluant A and 0.1 M sodium phosphate as eluant B (both at pH 7.0). Diluted samples prepared as described previously were loaded on the HPLC autosampler (Oellingrath et al. 1990). The system was operated at a flow rate of 1 mL/min. Absorbances were read at 420 nm. The linear gradient was 0-100% B to A during 15 min and total run time was 25 min for each sample. Peak areas were used for quantitative calculations for myoglobin and hemoglobin concentration.

Myoglobin and hemoglobin concentration were determined from HPLC data using appropriate standard curves. The myoglobin standard curve was prepared using lyophilized equine myoglobin powder (0.1 – 1.2 mg/ml), in 0.1 M phosphate buffer pH 7.0. The standard curve regression equation was:

$y = 15.45x - 2.13$ ($R^2 = 0.997$) where,

y= absorbance reading @ 420 nm; x = Concentration of myoglobin.

The hemoglobin standard curve was similarly prepared using lyophilized bovine hemoglobin powder similar to myoglobin. The standard curve regression equation for hemoglobin was:

$y = 57.17x - 0.236$ ($R^2 = 0.994$) where,

y = absorbance reading @ 420 nm; x = concentration of hemoglobin

Total pigment extracted in phosphate buffer

Total pigment as phosphate extraction was determined to calculate total myoglobin and hemoglobin. Finely minced beef samples (3g) were diluted with cold 25 mL of 0.04 M sodium phosphate buffer (pH 6.8) in 50 mL polypropylene tubes (Trout 1989). The samples were mixed with a glass rod and the tubes were kept in ice for 1 hr. This tube was then centrifuged at 21,500g for 30 min at 2°C. The supernatant was collected and absorbance was read at 525 and 700 nm spectrophotometrically using a Model UV-2100 UV-VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan) against blank phosphate buffer. Pigment concentration (mg/g muscle) was calculated using the following equation:

Total Myoglobin Pigment (mg/g) = $(A_{525} - A_{700})$ x 2.303 x dilution factor, where A525 was the isobestic point, i.e. the point on the visible spectra where all myoglobin (oxymyoglobin, deoxymyoglobin and metmyoglobin) have equal absorbance. Thus pigment concentration can be determined by a single

absorbance measurement at 525 nm. Pigment concentration was corrected for turbidity by subtraction of the absorbance value of the solution at 700 nm.

Total pigment extracted in acidified acetone

Total pigment as acid hematin was determined using the acidified acetone extraction of Hornsey (1956), with slight modification. Reagent used was acetone b (prepared by mixing 20 mL concentrated HCl to a final volume of 100 mL with deionized water). The diluted HCl was then transferred to a 1 liter volumetric flask, and brought to volume with acetone. The extraction procedure was conducted in dim light to minimize pigment fading. Minced beef samples (2g) were weighed into pyrex tubes, 9.0 mL acetone-b was added and samples were macerated using a glass rod. The tubes were capped and allowed to stand for 1 hr before filtering through Watman 42 filter paper. Filtrate absorbance was read at 640 nm spectrophotometrically (Spectronic 21D, Milton Roy, Rochester, NY) against a blank that contained all the reagents except beef sample, and total pigment was calculated using extinction coefficient from Hornsey (1956):

ppm total pigment = A_{640} x 680

Sensory Analysis

A sensory panel of 6 people was trained for descriptive analysis of beef muscles flavor profile. They were trained especially for liver flavor along with some other flavors like rancid, metallic and normal beef flavor. The panelists were trained by providing standard samples for each sample (Meilgaard et al.

1991). For liver flavor, beef liver was cooked to an internal temperature of 71°C and served. For rancid or oxidized flavor, beef steaks were cooked to the same temperature (as above) on the previous day, and kept at 2°C and served the next day after warming in microwave for 1 min. For beef flavor, fresh beef top loin steaks were cooked and served. All flavors were evaluated for intensity using a 5-point category scale where 1 = no flavor; 2 = slightly intense flavor; 3 = moderately intense flavor; 4 = very intense flavor; and 5 = extremely intense flavor. The training was conducted in two sessions. In the first session, panelists were familiarized with the 5-point scale and its usage. In the second session, cooked beef samples were evaluated for intensity of different flavors. Group discussion was conducted regarding sample flavor attributes. For sample evaluation five different beef chuck muscles (IF, TM, LD, SERR, and SS) were cooked to two different temperature (71 and 82°C) on an electric grill preheated to 177°C (Circulon Hi-flow system). Temperature was observed using a digital thermocouple thermometer (Atkins Temptec, Gainesville, FL). Panelists were seated in individual sensory booths equipped with red lights to prevent color biasedness among samples. Each panelist was served with samples, distilled water and unsalted crackers to cleanse the palate between two samples. Panelists' responses were recorded by the SIMS 2000 (Sensory Information Management System) software on a PC monitor in each booth. Rotation plan for SIMS 2000 has been shown in **Appendix D**. For statistical analysis, category scale was assigned numerical values, where 1 = no flavor; 2 = slightly intense flavor; 3 =

moderately intense flavor; 4 = very intense flavor; and 5 = extremely intense flavor. Data were analyzed using STATISTICA (Statsoft Inc., Tulsa, OK) software for mean values and comparison of means by analysis of variance (ANOVA).

Thiobarbituric Acid (TBA) Method

Thiobarbituric acid reactive substances (TBARS) were measured as described by Buege and Aust (1978). Duplicate samples (0.5 g) were weighed in two tubes with 2.5 mL stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis, MO), 15% TCA (Mallinckrodt Baker Inc., Paris, KY) and 1.0 N HCL. Sample tubes were heated in hot boiling water bath for 10 min to develop pink color and then cooled under running tap water. Samples were centrifuged (Sorvall Instruments, Model RC 5C, Dupont, Wilmington, DE) at 12,465xg for 25 min. Absorbance of supernatant was read at 532 nm using spectrophotometer against a blank sample having all reagents except the beef sample. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Sinhuber and Yu 1958). The MDA concentration was converted to TBA number (mg MDA/Kg meat sample) as shown below:

1. TBA # (mg/kg) = sample A_{532} X (1M MDA chromagen / 1.56×10^5) X [(1 mole / L) / M] X (0.003L / 0.5g meat) X (72.07g MDA / mole MDA) X (1000g / kg)
2. TBA # (ppm) = sample A_{532} X 2.77

Statistical Analysis

Analysis of variance was done on the data collected for each experiment using the MANOVA procedure of STATISTICA software (Statsoft Inc., Tulsa, OK). Fisher's least significant difference (LSD) test was done for post hoc mean comparison when $p<0.05$. Treatment means for sensory values and TBA values were also calculated using STATISTICA software.

RESULTS

Color and pigment concentration of five select grade beef chuck muscles

Mean myoglobin concentration ranged from 9.26±1.05 to 12.51±0.66 mg/g of muscle. The *serratus* muscle had significantly lower myoglobin concentration (9.26 mg/g) than other beef chuck muscles (Table 1, Figs. 4-8). Mean hemoglobin concentration were low (less than 2% of total heme pigment) ranging from 0.19±0.02 to 0.26±0.02 mg/g of muscle and not significantly different among the 5 muscles (select grade) of the beef chuck (Table 1, Figs. 4-8). *Teres major* and *serratus vebtralis* had relatively lower myoglobin and total heme pigment content (mg pigment/g muscle) than other muscles (Table 1), but the differences were not significant. *Infraspinatus* and *serratus ventralis* had higher levels of total pigment (Table 1) by phosphate extraction method, as follows: [*infraspinatus* (5.4 mg/g), *longissimus dorsi* (5.0 mg/g), *supraspinatus* (5.0 mg/g), *serratus ventralis* (5.7 mg/g), *terres major* (3.6 mg/g)]. However, statistical analysis showed that these differences were not significant.

There was no valid information obtained for processing condition (electrical stimulation or ES) for beef chuck muscles with regard to incidence of liver off-flavor. However total pigment concentrations was measured on all five beef chuck muscles from all three plants A, B, and C. The experiment was designed to compare muscles from processing plant A using ES to remove blood and plant B without ES. But at the conclusion of the experiment, it was learnt that both plants (A and B) used ES. Thus the original comparison was with and w/o could not be made. To compensate a single animal (commercial cow) was harvested at plant C (the USU meat lab harvest facility) and all five beef chuck muscles described previously, were sampled from this animal. As shown in Table 2, there was no significant difference in pooled mean pigment content from plant A and B because both plants used ES. The older animal from plant C had a numerically higher total pigment content than select grade animals from plants A and B. But this results were not statistically significant probably because sample size (n) was only 2 (2 samples from one animal). However, sensory panel results indicated that the infraspinatus muscle from the older animal (plant C) had a definite liver off-flavor. Because of this observation, a second experiment was conducted to compare effect of animal age (young = select grade; old = commercial grade) on IF muscle characteristics and sensory panel acceptability.

Table 1. Mean (± SD) for total myoglobin (Mb) and hemoglobin (Hb) by HPLC, Hunter color values, total pigment by phosphate extraction, and total hematin by acidified acetone extraction for raw select grade beef chuck muscles

Muscle	Mb (mg/g)	Hb (mg/g)	Hunter color values			Total pigment (mg/mL	Total hematin (ppm)
			L*	a*	b*		
IF	12.51±0.66	0.25±0.06	35.06±1.29	9.67±3.34	14.08±2.47	5.42±1.27	168.5±11.52
TM	11.05±0.84	0.26±0.02	36.02±5.87	9.81±3.87	13.36±2.49	3.60±0.25	133.92±33.89
LD	11.03±1.02	0.19±0.02	37.47±1.52	8.58±5.68	13.81±1.93	4.97±0.16	142.53±38.37
SERR	9.26±1.05	0.20±0.06	32.81±5.86	11.01±7.78	14.45±5.86	5.71±1.49	155.6±23.56
SS	11.36±1.03	0.22±0.09	33.31±3.27	8.88±5.54	13.69±4.24	5.01±0.30	141.13±24.87
p-value	0.02	NS	NS	NS	NS	NS	NS

IF = *infraspinatus*; TM = *teres major*; LD = *l. dorsi*; SERR = *serratus*; SS = *supraspinatus*; L* = lightness (greater L* value indicates a lighter color); a* = redness (greater a* value indicates a redder color); and b* = yellowness (greater b* value indicates a more yellow color); NS = not significant

Table 2. Mean (± SD) of total pigment pooled among five beef chuck muscles from three different processing plants

Statistica General Manova*	Means (± standard deviation)	
Plant	Total Pigment (mg/g)	N
A (select grade)	5.21±1.17	8
B (select grade)	5.06±1.17	16
C (commercial grade)	7.12±0.08	2

*A and B = with electrical stimulation; C = w/o electrical stimulation

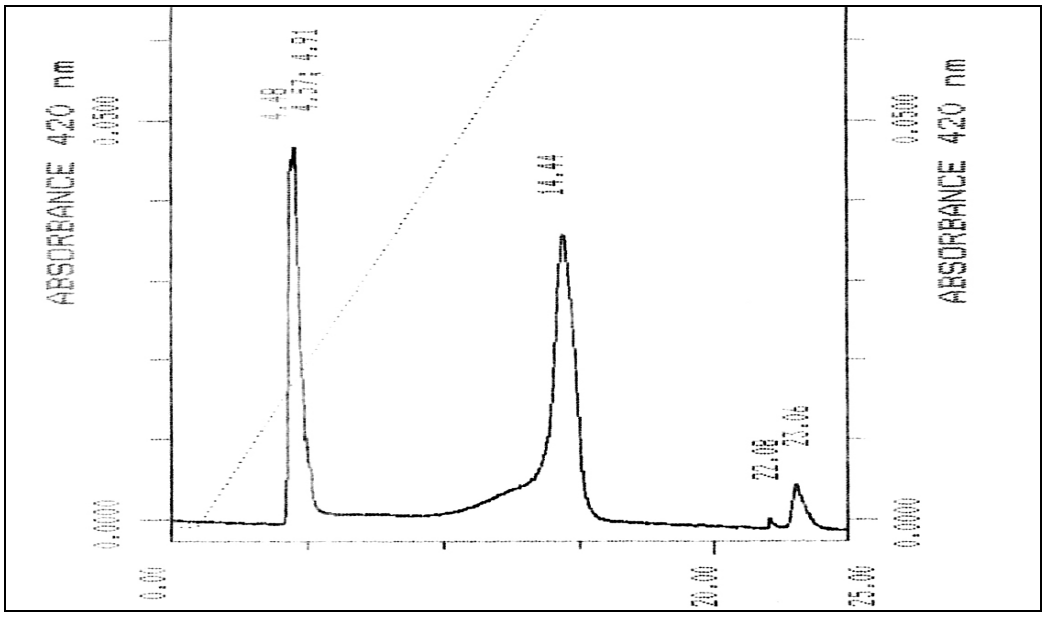

Figure 4. HPLC chromatogram for myoglobin and hemoglobin of select grade *infraspinatus* muscle

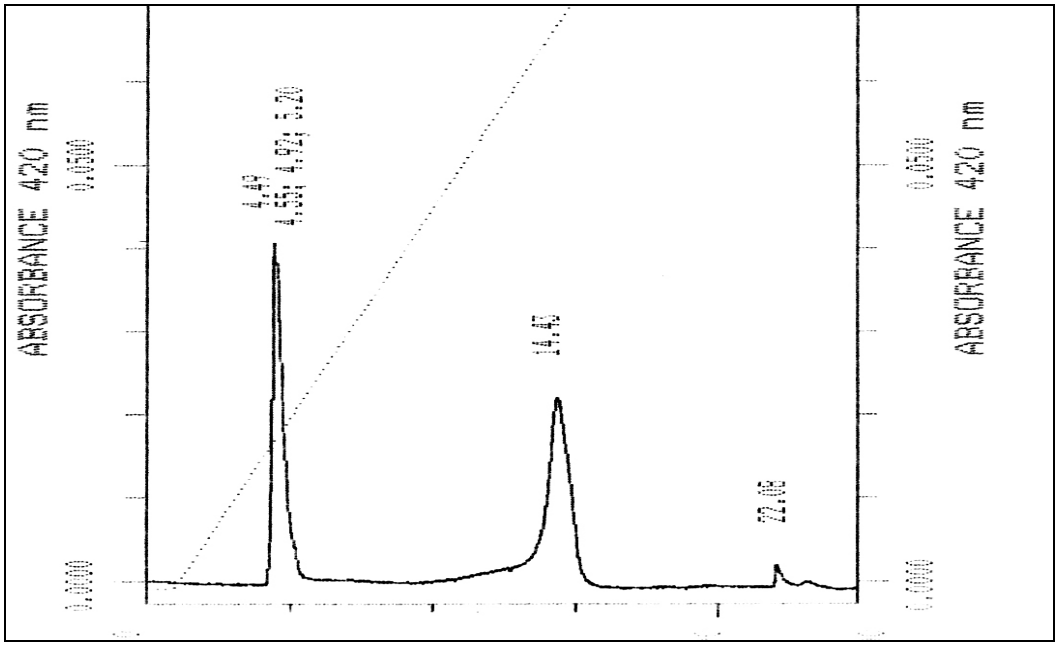

Figure 5. HPLC chromatogram for myoglobin and hemoglobin of select grade *teres major* muscle

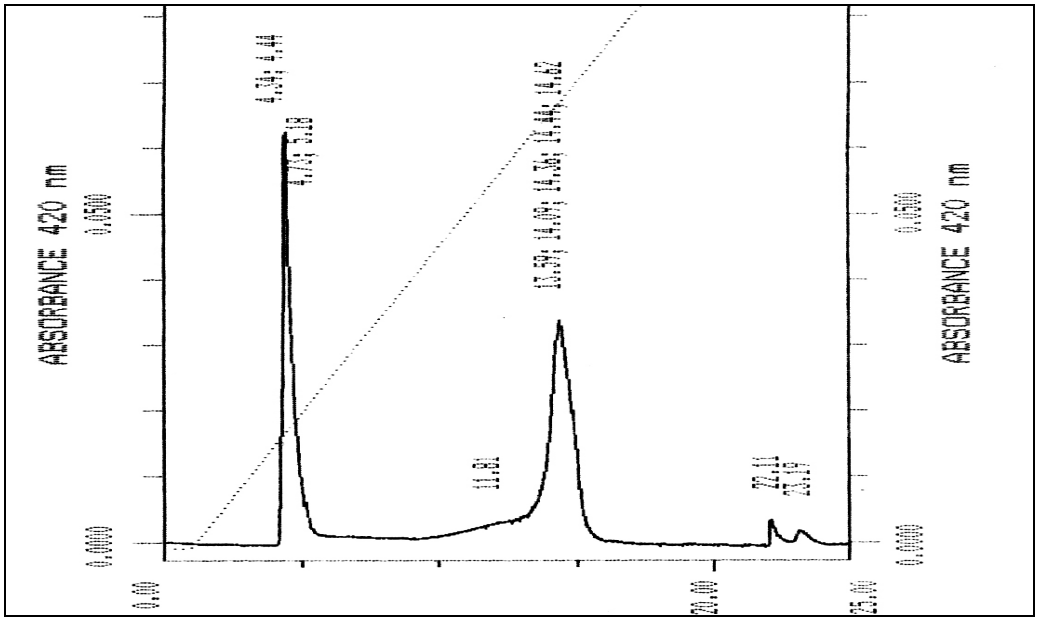

Figure 6. HPLC chromatogram for myoglobin and hemoglobin of select grade *l. dorsi* muscle

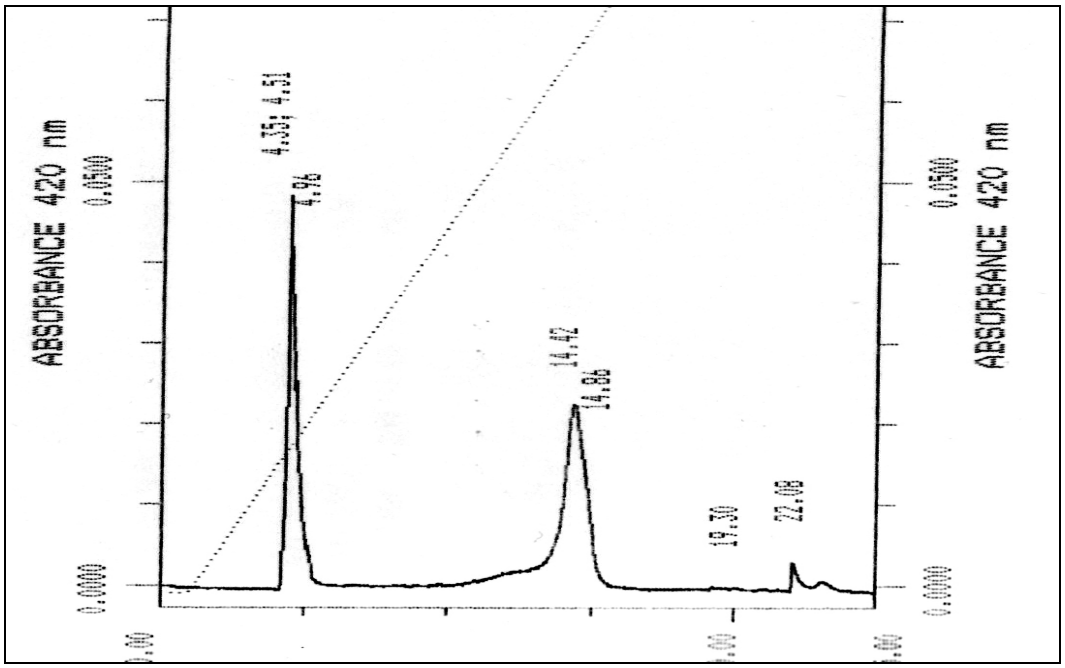

Figure 7. HPLC chromatogram for myoglobin and hemoglobin of select grade *supraspinatus* muscle

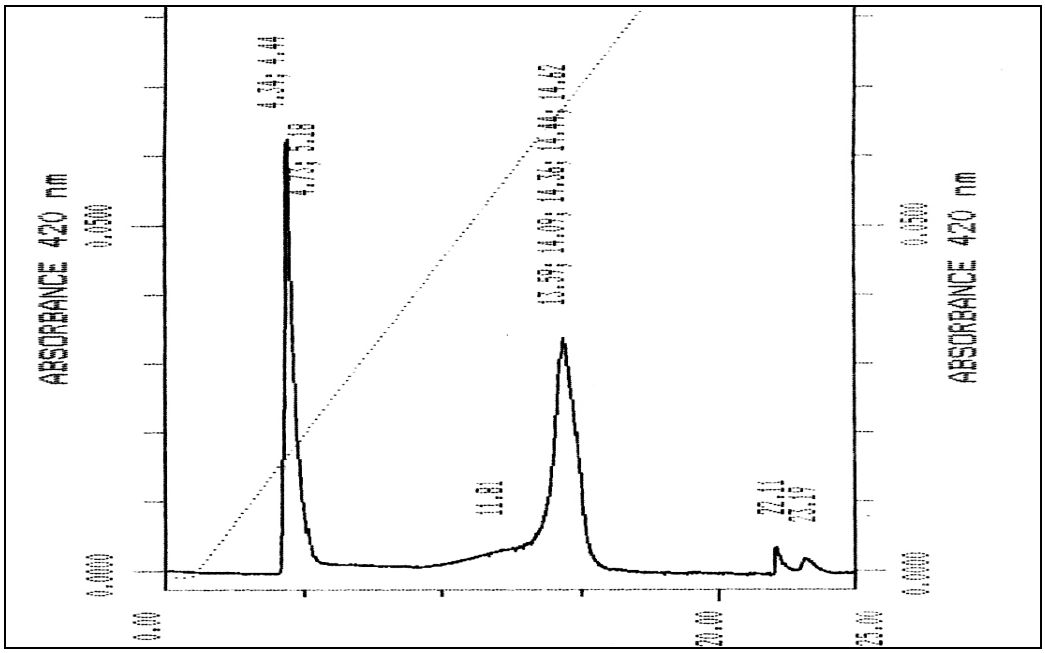

Figure 8. HPLC chromatogram for myoglobin and hemoglobin of select grade serratus muscle

Peak area absorbances were used in standard equation to measure the protein concentration.

Sensory analysis and TBA values

As shown in Table 3, after cooking, *infraspinatus* steaks had highest ($P < 0.05$) scores for liver flavor (2.08), on a 1-5 scale where 2 = slight liver flavor. Other liver flavor scores were 1.6 (*serratus*), 1.3 (*supraspinatus*), 1.1 (*longissimus*), and 1.0 (*teres major*). Scores were low (1.4 or less) and not significantly different among select grade muscles for rancid and metallic off-flavors. Beef flavor sensory scores were 3.2 – 3.6 (moderate), and were not different among muscles or carcass grades. Rancid flavor scores were significantly increased from 1.34±0.65 to 1.58±0.84 as internal cook temperature

increased from 71 to 82°C but mean TBA values as a measure of rancidity (0.25±0.15 and 0.29±0.13, respectively) were not affected by cook temperature (Table 4). However, sensory panel beef, metallic and liver flavor scores were not significantly affected by cook temperature. Detailed information and individual scores of flavor profile test is reported in **Appendix A**.

Table 3. Sensory panel scores of beef chuck muscles cooked to 71˚C

Muscle Type	Beefiness	Rancid	Metallic	Livery
IF	3.41±0.97	1.52±0.79	1.39±0.73	2.08±1.00a
LD	3.33±0.88	1.17±0.50	1.11±0.31	1.11±0.31b
SERR	2.97±0.75	1.47±0.67	1.33±0.70	1.33±0.60ab
SS	3.42±0.86	1.33±0.62	1.04±0.20	1.63±0.90a
TM	3.33±0.88	1.56±0.96	1.28±0.56	1.06±0.23b
LSD	NS	NS	NS	0.51

¥Means with a same letter are not significantly different ($p<0.05$); for muscle description see Table 1; LSD = least significant difference; NS = non significant

Table 4. Pooled mean (±SD) values for Sensory score for rancid and TBA # for beef chuck muscles cooked to 71 & 82°C

Temperature (°C)	Rancid score	TBA #
71	1.34 ± 0.65b	0.25 ± 0.15
82	1.58 ± 0.84a	0.29 ± 0.13
LSD	0.24	NS

DISCUSSION

The *infraspinatus* (beef chuck flatiron steak) muscle has a growing market as a restaurant grade steak **(**Morgan et al. 1991; Neely et al. 1998**).** This study showed that there were significant differences in pigment content and sensory characteristics among beef chuck muscles. In this study the beef chuck *serratus* muscle had significantly lower myoglobin content than IF or other chuck muscles as measured by HPLC. The IF was observed to be more susceptible to liver off-flavor as compared to other select grade beef chuck muscles. It possessed slight liver off-flavor (2.08, where 2 = slight liver flavor) but significantly higher than other beef chuck muscles. No effect of electrical stimulation and no significant differences among select grade muscles were observed for color, pigment, or hematin content. Our findings were in accordance with Yancey et al. (2006) who found that liver-like flavor was a complex trait and the association with meat pigment content was relatively low.

CONCLUSION

A slight to moderate liver flavor was detected by some (but not all) panelists in cooked *infraspinatus* (flat-iron) steaks from select grade muscles. No effect of electrical stimulation was observed on muscle pigment content or sensory scores because all samples from processing plants A and B were subjected to ES treatment and thus residual blood content was low and similar in all samples. Sensory panel rancid flavor scores were also slightly but significantly increased with increased cooking temperature (71 and 82°C) but

instrumental measurement of rancidity (TBA values) were not affected by cooking temperature.

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CHAPTER V

PACKAGING, ANTIOXIDANT TREATMENT, AND ANIMAL AGE EFFECTS ON SENSORY CHARACTERISTICS OF BEEF INFRASPINATUS MUSCLE

INTRODUCTION

Age of the animal is an important factor that affects quality of meat in general and flavors in particular (Morgan et al. 1991). Diet of the animal also plays a very vital role in ultimate meat quality (Crouse et al. 1984; Liu et al. 1995; Mandell et al. 1998; Muir et al. 1998). Off-flavors which are presently in serious discussion are sour/grassy and liver-like. Meisinger and others (2006) suggested that liver-like off-flavors were specific to individual animals, and that pH and heme iron are not strongly related to off-flavor notes. Miller (2001) reported that beef cuts with higher levels of myoglobin, higher degree of doneness or with higher degree of lipid oxidation typically express liver-like or metallic flavors. If liver flavors are indeed products of lipid oxidation in beef muscles, prevention may be possible by antioxidant injection combined with anaerobic packaging. Lipid oxidation and rancidity of steaks or ground beef can be inhibited by vacuum packaging or low oxygen modified atmosphere packaging with 0.4% carbon monoxide (John et al. 2004, 2005).

Based on the above findings and hypotheses, an experiment was designed to compare the *infraspinatus* muscles from select grade (young; <30 months old) and commercial grade animals (old; >42 months old) using HPLC for total hemoglobin content, total meat pigment using acetone extraction and

phosphate extraction methods, instrumental (L*, a*, and b*) as well as visual color (purple, red, and brown color intensity) inspection, and sensory evaluation for flavor by a trained panel followed by determining thiobarbituric acid (TBA) number to evaluate level of rancidity. Objective 2 of this study was to evaluate packaging and antioxidant treatments to prevent liver and sour/grassy off-flavor development. The packaging treatments included 80% O_2-MAP (modified atmospheric packaging), 0.4% carbon monoxide-MAP, or PVC (polyvinyl chloride) overwrap, after injection enhancement of raw steaks in antioxidant solutions of 0.3% sodium tripolyphosphate (STP) and 500 ppm ascorbic acid.

Injection of fresh beef steaks and roasts with solution of ascorbic acid and STP is now permitted by the USDA. Thus, "enhancement" injection of fresh beef is increasingly used, particularly in MAP products. Injection of beef loin strips with phosphate, lactate, and sodium chloride improved beef tenderness and juiciness (Vote et al. 2000). Mancini and others (2005) used enhancement solutions of lactate and rosemary to improve strip loin steak color stability, and reported that lactate-enhanced products were darker but had a more stable red color.

To prevent or reduce liver flavor development, it is proposed in this study to inject STP and ascorbic acid, in combination, as antioxidants into beef *infraspinatus* (IF or flat iron) steaks in 0.4% CO-MAP, or aerobic packaging in 80% oxygen-MAP, or in standard oxygen-permeable PVC film wrap.

MATERIALS AND METHODS

Color Measurement

The color of the *infraspinatus* muscle was measured using a Hunter Lab Miniscan portable colorimeter (Reston, VA). The instrument was standardized using white and black standard plates covered with the same plastic film used for meat color measurements. CIE L* (lightness), a* (redness) and b* (yellowness) values were measured on meat samples using illuminant D65. The a* value represents redness so brighter red beef muscle samples, had higher a* values. Hue angle was also calculated as arctangent (b*/a*), where hue angle values near zero are red and higher values are increasingly less red and more yellow.

HPLC

Sample preparation: Lean beef muscle (1000 g) was ground through a fine plate (3 mm diameter pore size) of a Hobart grinder (Model model 4152; Hobart Mfg. Co., Troy, OH). Ground beef samples (10 g) were diluted with 30 mL of 0.1 M sodium phosphate buffer pH 7.0 in a stomaching bag, and mixed for 1 min. at high speed using a Colworth Stomacher 400 Model BA7021 (London, UK). The entire mixture was transferred to a 50 mL polypropylene tube and centrifuged (Sorvall Instruments, Model RC 5C, Dupont, Wilmington, DE) at 21,500g for 30 min at 2-5˚C. The supernatant was then filtered through a 0.45-micron membrane filter disc (Millipore, Billerica, MA). A 2 mL portion of filtered solution was diluted to 10 mL with 0.1 M sodium phosphate buffer. A

few grains of potassium cyanide were added to maintain the heme pigments in their cyano-ferric form. Filtered samples (40 µl) were transferred to a 100 µl plastic, tappered bottom vial. The vial was placed on the holding rack of the HPLC autosampler. The autosampler was set to withdraw 25 µl from each sample vial for pigment separation and quantitation.

Instrument description: A Beckman System Gold HPLC unit (Beckman Instruments, Inc., Fullerton, CA)) with Autosampler 507 and UV detector instrument was used to run extracted beef samples for determining hemoglobin and myoglobin content. A hydrophobic column (Bio-Gel TSK Phenyl-PW 7.5 cm x 7.5 mm; Tosoh Biosciences LLC, Montgomeryville, PA, USA) was used. The column was operated with 1.7 M ammonium sulphate, 0.1 M sodium phosphate as eluant A and 0.1 M sodium phosphate as eluant B (both at pH 7.0). Diluted samples prepared as described previously were loaded on the HPLC autosampler (Oellingrath et al. 1990). The system was operated at a flow rate of 1 mL/min. Absorbances were read at 420 nm. The linear gradient was 0-100% B to A during 15 min and total run time was 25 min for each sample. Peak areas were used for quantitative calculations for myoglobin and hemoglobin concentration.

Myoglobin and hemoglobin concentration were determined from HPLC data using appropriate standard curves. The myoglobin standard curve was prepared using lyophilized equine myoglobin powder (0.1 – 1.2 mg/ml), in 0.1 M phosphate buffer pH 7.0. The standard curve regression equation was:

$y = 15.45x – 2.13$ ($R^2 = 0.997$) where,

y= absorbance reading @ 420 nm; x = Concentration of myoglobin

for IF muscle sample.

The Hemoglobin standard curve was similarly prepared using lyophilized bovine hemoglobin powder similar to myoglobin. The standard curve regression equation for hemoglobin was:

$y = 57.17x - 0.236$ ($R^2 = 0.994$) where

y = absorbance reading @ 420 nm; x = concentration of hemoglobin

Total pigment extracted in phosphate buffer

Finely minced beef samples (3g) were diluted with cold 25 mL of 0.04 M sodium phosphate buffer (pH 6.8) in 50 mL polypropylene tubes (Trout 1989). The samples were mixed with a glass rod and the tubes were kept in ice for 1 hr. Tubes were then centrifuged at 21, 500g for 30 min at 2C. The supernatant was collected and absorbance was read at 525 and 700 nm spectrophotometrically using a Model UV-2100 UV-VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan) against blank phosphate buffer. Pigment concentration (myoglobin + hemoglobin mg/g muscle) was calculated using the following equation:

Total soluble heme (mg/g) = (A525 – A700) x 2.303 x dilution factor,

where A525 was the isobestic point, i.e. the point on the visible spectra where myoglobin or hemoglobin forms (oxy, deoxy, or met) have equal absorbance. Thus pigment concentration can be determined by a single absorbance

measurement at 525 nm. Soluble pigment concentration was corrected for turbidity by subtraction of the absorbance value of the solution at 700 nm.

Total pigment extracted in acidified acetone

Total pigment as acid hematin was determined using the acidified acetone extraction of Hornsey (1956), with slight modification. Reagent used was acetone b (prepared by mixing 20 mL concentrated HCl to a final volume of 100 mL with deionized water). The diluted HCl was then transferred to a 1 liter volumetric flask, and brought to a volume with acetone. The sample preparation and procedures were conducted in dim light to minimize pigment fading. Minced beef samples (2g) were weighed into pyrex tubes, 9.0 mL acetone-b was added and samples were macerated using a glass rod. The tubes were capped and allowed to stand for 1 hr before filtering through Whatman 42 filter paper. Filtrate absorbance was read at 640 nm spectrophotometrically using a Model UV-2100 UV-VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan) against a blank that contained all the reagents except beef sample, and total pigment was calculated using an extinction coefficient from Hornsey (1956):

ppm total pigment = A_{640} x 680

Antioxidant and packaging treatment

The experiment was a factorial design, with three packaging methods (CO-MAP, 80% oxygen MAP, PVC wrap), and two antioxidant treatments (control non-injected or injected with a solution of 0.3% STP + 500 ppm

ascorbic acid). *Infraspinatus* steaks were injected to obtain a 6% weight gain. Modified atmosphere packaging was done as described by John and others (2005). Individual steaks were placed in vacuum bags (15 x 25 cm). Ambient air was removed by the vacuum cycle, and the desired atmosphere was flushed into the bag. Packages were heat sealed. Cylinders certified to contain the desired gas composition (0.4% CO + 30% CO_2 + 69.6% N_2; or 80% oxygen + 20% CO_2) were obtained from Air Gas Corp, Salt Lake City, UT). Initial packages from each packaging run were tested to verify that oxygen concentrations are less than 10 ppm in CO-MAP packages, and 80 ± 2% in high oxygen packaging, using an Illinois Instruments Oxygen Analyzer (model 3500; Illinois Instruments, Ingleside, IL, USA). Packaged steaks were stored at 2°C for a period typical for each packaging method, as follows: CO-MAP for 21 days, 80% oxygen for 10 days, and PVC wrap for 5 days. At the end of the storage period, 2 steaks were used for measurement of visual and Hunter color measurements as described earlier. The remaining steaks were cooked to an internal temperature of 74°C (medium).

Sensory analysis

A sensory panel of 6 people were trained for descriptive analysis of beef muscle flavor profile and tenderness. Panelists were trained to recognize intensity of normal beefy flavor as well as off-flavors including rancid, metallic, and sour/grassy. The panelists were trained by providing standard samples for each sample (Meilgaard et al. 1991). For liver flavor, beef liver was cooked to

71˚C internal temperature and served warm. For rancid or oxidized flavor, beef steaks were cooked to 71˚C on the previous day, kept at 2˚C and served the next day after warming in a microwave for 1 min.. For beef flavor, fresh beef top loin steaks were cooked as described previously and served. All flavors were evaluated for intensity using a 5-point category scale where 1 = no flavor/tenderness/toughness; 2 = slightly intense flavor/tenderness/toughness; 3 = moderately intense flavor/tenderness/toughness; 4 = very intense flavor/tenderness/toughness; and 5 = extremely intense flavor/tenderness/toughness. The training was conducted in two sessions. In the first session, panelists were familiarized with the 5-point scale and its usage. In the second session, cooked beef samples were evaluated for intensity of different flavors. Group discussion was conducted regarding sample flavor, tenderness, and toughness attributes. *Infraspinatus* muscle from young select and old commercial grade animal were cooked to an internal temperature of 74°C on an electric grill preheated to 177°C (Circulon Hi-flow system) and temperature was monitored using a thermocouple thermometer (Atkins Temptec, Gainesville, FL). Panelists were seated in individual sensory booths equipped with computers. Samples were coded (balanced and randomized) prior to serving. Distilled water and unsalted crackers were available to cleanse the palate between samples. Panelist responses were recorded by the SIMS 2000 (Sensory Information Management System) software (version 6) and were analyzed using STATISTICA (Statsoft Inc., Tulsa, OK) software for mean values and comparison of means by analysis of variance (ANOVA). For statistical analysis,

category scale was assigned numerical values, where 1 = no flavor; 2 = slightly intense flavor; 3 = moderately intense flavor; 4 = very intense flavor; and 5 = extremely intense flavor.

Warner Bratzler Shear Force (WBSF) test

The firmness/tenderness of samples was measured using a Warner-Bratzler Shear Press (G.R. Electric Man. Co., Manhattan, KS) to measure the force (kg) applied to completely shear the sample (Quinton et al. 1997). Each steak was cooled to room temperature, and three 1.27-cm-diameter cores were removed from each steak parallel to the muscle fiber orientation using a manual-coring device. A single peak shear force measurement was obtained for each core using the WBSF instrument (G-R Elec. Mfg. Co., Manhattan, KS). Peak WBSF values from each steak were averaged for statistical purposes (Roeber et al. 2005).

pH

The pH values for each sample were measured by adding 90 mL deionized water to 10 g sample. The samples were then thoroughly mixed and passed through Whatman filter paper no. 2 (Fisher Scientific, Salt lake City, UT). The pH of the filtrate was measured using a pH meter calibrated at pH 4.0 and 7.0 (Fisher Chemicals, Fair Lawn, NJ).

Thiobarbituric acid (TBA) method

Thiobarbituric acid reactive substances (TBARS) were measured as described by Buege and Aust (1978). Duplicate samples (0.5 g) were weighed in two tubes with 2.5 mL stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis, MO), 15% TCA (Mallinckrodt Baker Inc., Paris, KY) and 1.0 N HCL. Sample tubes were heated in hot boiling water bath for 10 min to develop pink color and then cooled under running tap water. Samples were centrifuged (Sorvall Instruments, Model RC 5C, Dupont, Wilmington, DE) at 12,465xg for 25 min. Absorbance of supernatant was read at 532 nm using a spectrophotometer, against a blank sample having all reagents except the beef sample. The malonaldehyde (MDA) concentration was calculated using a chromagen extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Sinhuber and Yu 1958). The MDA concentration was converted to TBA number (mg MDA/Kg meat sample) as shown below:

3. TBA # (mg/kg) = sample A_{532} X (1M MDA chromagen / 1.56×10^5) X [(1 mole / L) / M] X (0.003L / 0.5g meat) X (72.07g MDA / mole MDA) X (1000g / kg)

4. TBA # (ppm) = sample A_{532} X 2.77

Statistical design and analysis

This experiment is a factorial design (3x2x2) with three packaging methods (PVC, 80% O2-MAP, 0.4% CO-MAP), two antioxidant treatments (with or without injection of 0.3% STP + 500 ppm ascorbate), and two

grades/age (select grade or young, and commercial grade or old).

Analysis of variance was done on the data collected for each experiment using the MANOVA procedure of STATISTICA (Statsoft Inc., Tulsa, OK). Fisher's least significant difference (LSD) test was done for post hoc mean comparison when $p<0.05$. Treatment means for sensory values and TBA values were also calculated using STATISTICA software.

RESULTS AND DISCUSSION

In a preliminary experiment, cooked IF steaks from a single commercial grade cow exhibited moderate liver flavor. Thus a further study was done on IF muscles from six commercial cows, cooked to 74˚C. Data obtained from this study is included in **Appendix C**.

Raw muscle sample (total myoglobin, hemoglobin, total hematin and Hunter color values)

Myoglobin and hemoglobin content of IF muscles of select and commercial grade cattle were measured by HPLC (Fig. 5), following the procedure described by Oellingrath and others (1990). Myoglobin and hemoglobin eluted at 5 and 21 min. respectively for standard solution and extracts of IF muscles. A third large peak with elution time of 14 min was also observed in all IF samples. The peak at 14 min. was not cytochrome C, the major hemoprotein in muscles other than myoglobin and hemoglobin (Fisher et al. 1973). One possibility was that the 14 min. peak was a myoglobin dimer formed by disulfide linkages between myoglobin molecules. However, incubation of IF

muscle extracts with excess sodium dithionite did not alter the HPLC chromatogram. Thus the identity of 14 min peak remained unknown. No significant difference was observed in myoglobin or hemoglobin content between old (commercial) and young (select) *infraspinatus* muscle.

In this study the HPLC method was useful to identify myoglobin and hemoglobin but HPLC pigment quantitation was not as sensitive as the other methods used in this study (Fig. 5).

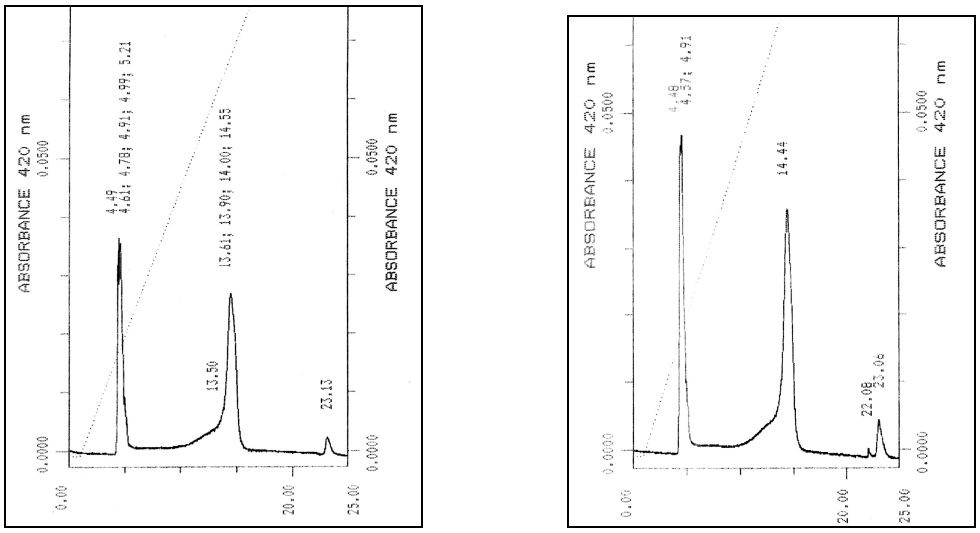

Figure 9 HPLC chromatogram for select grade IF and commercial grade IF muscles for myoglobin and hemoglobin

(Mb, elution time 4-5 min; Hb, elution time 21-22 min; A = Select grade IF muscle; B = Commercial grade IF muscle)

Effect of grade (commercial or select) on total pigment, hematin, and color before treatment

Raw muscle pigment concentration before antioxidant treatment and packaging was measured by phosphate extraction (Trout 1989) and as acidified

acetone extractable hematin (Hornsey 1956; Table 4). Total phosphate-extractable pigment in commercial grade *infraspinatus* (IF) muscle was higher ($p<0.05$) than select grade *infraspinatus* muscle (8.94 and 5.73 mg hemoprotein /g muscle, respectively; Table 4). Total hematin content was also higher ($p<0.05$) in commercial grade IF muscle compared to select grade samples (229 and 127 ppm, respectively). Color parameters a*, b*, and hue angle were also significantly different among commercial and select grade *infraspinatus* samples, with lower mean values for a*, b*, and hue angle for commercial than select grade IF samples (Table 4). Photos of IF muscles stored in three different packaging methods are added in **Appendix C**. Thus commercial grade IF muscle had higher total pigment and acid hematin content than select grade muscle in agreement with previous work (Smith et al. 1988; Renand et al. 2001).

Treatment and interaction effects on color parameters

The main and interaction effects of packaging and antioxidant treatment on Hunter color values of select and commercial grade IF muscles are shown in Table 6. The redness (a*) and hue angle values were significantly affected by packaging method (PVC, 80% O_2-MAP, and 0.4% CO-MAP) and by antioxidant treatment (with or without 0.3% sodium tripolyphosphate + 500 ppm ascorbic acid), and the interaction of packaging method with antioxidant treatment

There was a significant effect of packaging (pooled with antioxidant treatment) on a* values and hue angle (indicator of redness) of select grade

infraspinatus (IF) muscle (Table 7) with highest a* value and lowest hue angle value recorded for 0.4% CO-MAP packaging method. Packaging storage times were different by design as typical for each packaging method; 5, 10, 21 days for PVC, 80% O_2, 0.4% CO, respectively. After 5 days in PVC, 10 days in 80% O_2, or 21 days in 0.4% CO steaks maintained generally desirable red appearance, with a* values greater than 10 (not different from each other) for commercial grade IF steaks. The reason for high red color stability for samples in all packaging methods was probably because older (commercial grade) animals are typically pastured, resulting in higher muscle content of vitamin E and other antioxidants (Yang et al. 2002), thus more resistant to pigment oxidation and browning than are from the muscles from younger (select) animals.

Table 5. Mean (±SD) for total hemoglobin (Hb), pigment, hematin, and Hunter color values for commercial and select grade *infraspinatus* muscle

Treatment	Total Hb (mg/g) by HPLC	Total pigment (mg/g)	Total hematin (ppm)	L*	a*	b*	Hue angle
Old IF muscle (commercial grade)	0.45±0.09	8.64±2.06	229.54±23.83	32.81±6.76	7.90±1.96	8.77±2.53	49.22±1.03
Young IF muscle (select grade)	0.25±0.06	5.42±1.27	168.50±11.52	35.06±1.29	9.67±3.34	14.08±2.47	52.17±2.17
p-level	NS	0.019	0.000	NS	0.014	0.002	0.035

[a,b] values in columns with different letters are different ($p < 0.05$)
L* = lightness; a* = redness; b* = yellowness; hue angle = atan(b*/a*)
IF = *Infraspinatus* muscle

Table 6. Main and interaction effects (total n = 36) of packaging, antioxidants, and age on Hunter color values of raw select and commercial grade *infraspinatus* muscle

Effect	n	L*	a*	b*	Hue angle
Packaging[1]	12	S	S	S	S
Meat age (grade)[2]	18	NS	S	S	S
Antioxidant[3]	18	NS	NS	S	NS
Observation per sample	12	NS	NS	NS	NS
Packaging x antioxidant	6	S	S	S	S

L* = lightness; a* = redness; b* = yellowness; and hue angle = $\tan^{-1}$ (b*/a*) or atan(b*/a*)
[1]Samples were packaged in PVC-wrap, 80% O_2-modified atmospheric packaging (MAP), and 0.4% CO-MAP
[2]Beef samples were select grade (young) and commercial grade (old) *infraspinatus* muscle
[3]Antioxidants used were 0.3% sodium tripolyphosphate and 500 ppm ascorbic acid in combination
S = Significant at $p<0.05$
NS = Nonsignificant

Table 7. Hunter color mean values of select and commercial grade IF muscle as affected by packaging (PKG) method (pooled with antioxidant treatment)

PKG	Select grade IF muscle				Commercial grade IF muscle			
	L*	a*	b*	Hue	L*	a*	b*	Hue
PVC	35.93 ± 4.34	6.91 ± 1.22	12.57 ± 1.01	61.23±5.21	29.75± 5.86	14.72 ± 3.00	14.13 ± 2.04	44.13±4.54
80% O2-MAP	38.60 ± 2.89	7.21 ± 1.60	13.63 ± 1.59	62.15±6.73	34.55 ± 3.70	14.16 ± 3.33	16.62 ± 2.33	44.13±4.54
0.4% CO-MAP	34.89 ± 3.88	20.93 ± 1.45	14.65 ± 0.23	35.00±3.75	39.09 ± 6.43	17.75 ± 2.88	13.61 ± 2.89	44.13±4.54
p-level	NS	0.02	NS	0.00	NS	NS	NS	NS

*p-level significance at 0.05; NS = not significant; [a,b]Means with different letter within a column are different

Panel Evaluation

A six-member trained panel evaluated cooked IF samples for intensities of beef flavor and various possible off-flavors (metallic, rancid, liver, and sour/grassy) and also tenderness on a 5-point intensity scale where, 1 = no flavor/tenderness, and 5 = extremely intense flavor/tenderness. The pooled main effect of packaging significantly affected rancid flavor intensity ($p = 0.005$), and TBA values ($p = 0.0000$). Panel tenderness scores tended towards significance ($p = 0.069$) as affected by packaging method (Table 8). The pooled main effect of antioxidant injection significantly affected trained panel scores for tenderness and toughness ($p<0.05$) but other flavor attributes, WBS, and TBA number remained unaffected and nonsignificant. The interaction effect of packaging with antioxidant treatment (Table 9) significantly affected trained panel flavor scores and instrumental analysis of WBS and TBA. There was significant interaction of packaging and antioxidant treatment (Table 9) on beefiness, rancidity, tenderness, and toughness scores ($p<0.05$). The WBS and TBA number were also affected by treatment interactions ($p = 0.03$ and 0.0000, respectively). The mean TBA values were highest in PVC packaged samples (Table 10). Mean values for rancid flavor intensity were highest in 80% oxygen-MAP injected with antioxidant solution (Table 9) perhaps because the antioxidant solution was not deaerated, and so might contain oxygen that contributed to sample oxidation in some cases. There was no difference observed in rancid flavor scores for 0.4% CO-MAP with and without injection of antioxidants probably because this

packaging method was anaerobic and thus antioxidant treatment would have no additional antioxidant effect. In PVC, antioxidant treatment decreased the rancid flavor scores (Table 9). Liver flavor was not detected in these samples, despite high heme pigment content, but three of six samples exhibited moderate to very intense sour/grassy flavor. One sample was a dark cutter (pH 6.49), with bland or "unusual" beef flavor. IF steaks in CO-MAP for 21 days also had higher panel tenderness scores and lower scores for sour/grassy or rancid off-flavors than steaks in aerobic packaging.

Tenderness scores increased and toughness decreased when antioxidant treatment was used with PVC packaging (Table 9). A similar pattern was observed in instrumental analysis for tenderness by WBS and rancidity by TBA number.

In addition, there was significant difference among the mean values ($p<0.05$) for beefy and rancid flavor, tenderness and toughness for the sensory panel scores (Table 9) for commercial grade IF muscle. Beefy flavor was significantly increased when packaged in 0.4% CO-MAP with antioxidant injection as shown in Table 8. Rancid flavor decreased when IF muscles were packaged in 80% O2-MAP with antioxidant injection as compared to PVC and 80% O2-MAP without antioxidant injection. No significant difference in rancid flavor score was observed in anaerobically packaged (0.4% CO-MAP with and without antioxidant injection) IF muscle (Table 9). Tenderness scores were higher and toughness scores were reduced for IF muscles treated with antioxidant injection for all three packaging methods (Table 9). In agreement with sensory

data, Warner-Bratzler shear values were also significantly higher (samples were tougher) for muscles packaged without antioxidant injection. However, this was only true for samples in aerobic packaging. Samples in anaerobic packaging actually had higher WBS values for muscles packaged with antioxidant injection (Table 10). TBA values were higher for PVC followed by 80% O2-MAP and 0.4% CO-MAP packaging without antioxidant injection. Antioxidant injection significantly lowered TBA values of aerobically packaged muscles (PVC or 80% O2), but antioxidant injection had no effect on TBA values of anaerobically packaged steaks, i.e., those steaks in 0.4% CO-MAP (Table 10).

Table 8. Pooled treatment mean effect of packaging on sensory panel scores, Warner-Bratzler shear (WBS) and thiobarbituric acid (TBA) values

Packaging	Antioxidant	Beefiness	Metallic	Rancid	Livery	Sour/grassy	Tenderness	Toughness	WBS	TBA
PVC	***	2.53	1.00	1.20	1.03	1.80	2.40	2.26	2.70	0.85
80% O2-MAP	***	2.33	1.07	2.00	1.33	2.03	2.16	2.40	2.31	0.31
0.4% CO-MAP	***	2.60	1.03	1.80	1.23	2.13	2.23	1.70	2.80	0.46
Significance	***	NS	NS	0.01	NS	NS	NS	NS	NS	0.00

NS = Non-significant; Trained panel scores were on a 5-point scale, 1 = no flavor/tenderness, and 5 = extremely intense flavor/tenderness; WBS = Warner Braztler Shear values in Kg; TBA = Thiobarbituric acid number

Table 9. Mean sensory scores of commercial grade IF muscle as affected by the interaction of antioxidant treatment and packaging method

Treatment	Beefiness	Metallic	Rancid	Livery	Sour/grassy	Tenderness	Toughness
PVC	3.07±1.40a	1.00±0.00	1.27±0.60b	1.00±0.00	2.00±1.10	1.60±0.90b	3.07±1.50a
PVC + AO	2.00±1.20c	1.00±0.00	1.13±0.40b	1.07±0.30	1.60±0.90	3.13±1.50a	1.47±0.70c
80% O2-MAP	2.27±1.10ab	1.00±0.00	1.80±0.90ab	1.47±0.80	1.87±1.30	2.13±1.20b	2.67±1.30ab
80% O2-MAP + AO	2.40±0.90a	1.13±0.40	2.20±1.10a	1.20±0.60	2.20±1.10	2.20±1.10b	2.13±1.50bc
0.4% CO-MAP	2.20±0.90c	1.07±0.30	1.80±1.30ab	1.33±0.80	2.27±1.40	2.13±1.10b	1.93±1.10bc
0.4% CO-MAP + AO	3.00±0.70ab	1.00±0.00	1.80±1.10ab	1.80±1.10	2.00±0.80	2.33±0.70ab	1.47±0.90c
LSD	0.76	NS	0.7	NS	NS	0.81	0.89

Antioxidants (AO) = 0.3% sodium tripolyphosphate + 500 ppm ascorbic acid in combination
MAP = Modified atmospheric packaging
[a,b,c] Means within a column with the same are not different

Table 10. Mean Warner Bratzler Shear and TBA values of commercial grade IF muscle as affected by the interaction of antioxidant treatment and packaging method

Treatment	Warner Bratzler Shear values (Kg)	TBA #
PVC	3.15 ± 1.35^{ab}	1.01 ± 0.1^{a}
PVC + AO	2.24 ± 0.9^{c}	0.60 ± 0.1^{b}
80% O2-MAP	2.58 ± 0.6^{abc}	0.40 ± 0.1^{c}
80% O2-MAP + AO	2.08 ± 0.4^{c}	0.21 ± 0.01^{d}
0.4% CO-MAP	2.36 ± 0.9^{bc}	0.41 ± 0.1^{c}
0.4% CO-MAP + AO	3.21 ± 0.7^{a}	0.51 ± 0.1^{bc}
LSD	0.83	0.11

Antioxidant (AO) = 0.3% sodium tripolyphosphate + 500 ppm ascorbic acid in combination
MAP = Modified atmospheric packaging
a,b,c Means within a column with the same are not different

CONCLUSION

In conclusion, *infraspinatus* (IF) steaks from commercial grade (older) animals had higher sensory scores for several off-flavors (sour/grassy, liver-like) as compared to select grade IF muscle. Anaerobic packaging with CO-MAP and with antioxidant injection can be used to tenderize the commercial grade muscles and also maintain their characteristic beefy flavor and red color for at least 21 days. Moreover, liver flavor was not strongly associated with total heme pigment content in agreement with Yancey and others (2006). Other off-flavors were lower in anaerobically packaged steaks, compared to aerobic packaging.

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CHAPTER VI

SUMMARY AND CONCLUSIONS

Experiment 1 (Chapter IV) demonstrated the vulnerability of select grade *infraspinatus* (IF) towards livery off-flavor development. In experiment 2 (Chapter V) it was found that commercial grade IF muscle was prone towards sour/grassy off-flavor. Among beef chuck muscles studied (*infraspinatus*, *L. dorsi*, *teres major*, *serratus ventralis*, and *supraspinatus*) in part 1, the IF showed slight to moderate liver off-flavor incidence. Therefore, the IF was used in experiment 2 to evaluate packaging methods (PVC-wrap, 80% O_2-modified atmospheric packaging, and 0.4% CO-modified atmospheric packaging) with or without antioxidant injection (combination of 0.3% sodium tripolyphosphate + 500 ppm ascorbic acid) to possibly reduce off-flavors.

This study clearly demonstrated the benefits of anaerobic packaging (0.4% CO-modified atmospheric packaging) in keeping meat red and fresh for 21 days of storage. This was demonstrated by higher a* values and visual color (Appendix C), and lower TBA values for steaks packaged in 0.4% CO-MAP. Trained panel scores for beefiness were higher for anaerobic packaging as compared to aerobic packaging (PVC-wrap and 80% O_2-modified atmospheric packaging) even after 21 days of storage. The incidences of off-flavors like rancid and liver-like were also lower in anaerobic packaging.

The cause of liver off-flavor could not be solidly stated but was found in these experiments to vary among individual animal muscles and with animal age or grade.

In conclusion, liver off-flavor is a sporadic problem in beef chuck muscles and does not appear to be associated with any single parameter like residual blood or heme pigment content. The results of this study were in agreement with other researchers regarding liver off-flavor. In agreement with some previous studies, the IF muscles from commercial grade (older) animals was found to have higher sensory panel scores for sour/grassy off-flavors and contain higher total myoglobin and hemoglobin, total hematin, and darker color than select grade IF. Commercial grade IF muscles were also less tender and beefy flavored. The 0.4% CO packaging with antioxidant treatment proved to be useful to increase the tenderness and decrease sour/grassy off-flavors in commercial grade IF muscles, but no effects of packaging on liver flavor were found.

APPENDICES

APPENDIX A

DATA FOR CHAPTER IV

Data from total phosphate extractable pigment (myoglobin + hemoglobin) using Trout procedure

Select grade MUSCLE sample	Meat PLANT*	Total MB + HB (mg protein MB + HB per g of muscle)
IF	B	4.03
IF	B	4.03
IF	A	6.78
IF	A	4.27
IF	B	7.42
IF	B	5.37
IF	C	7.18
IF	C	7.06
IF	A	4.69
IF	A	4.28
IF	B	4.98
IF	B	5.06
IF	A	4.42
IF	A	4.42
IF	A	7.15
IF	A	5.7
LD	B	5.08
LD	B	4.86
SERR	B	7.87
SERR	B	4.44
SERR	B	5.27
SERR	B	5.27
SS	B	4.8
SS	B	5.23
TM	B	3.42
TM	B	3.78

***Meat plant A, B, C = Different meat processing plants in the vicinity.**

Data from acidified acetone total hematin done by Hornsey B method

Select grade muscle sample	Meat plant	Total hematin (ppm)
IF	B	178.8
IF	B	159.9
IF	C	157.2
IF	C	178
TM	B	101.5
TM	B	122.9
TM	C	170.9
TM	C	169
TM	C	105.3
LD	B	158.6
LD	B	163.9
LD	B	162.6
LD	C	85.1
SERR	B	174
SERR	B	173.9
SERR	B	121.2
SERR	C	167.8
SERR	C	141
SS	B	146.5
SS	B	142.7
SS	C	107.6
SS	C	167.2

***Meat plant B, C = Different meat processing plants in the vicinity.**

Sensory Panel scores (5-point intensity scale) for all five select grade beef chuck muscles

Muscle	Plant	Temp (°C)	Beefy	Metallic	Rancid	Livery
IF	A	71	3	2	1	2
IF	A	71	3	1	1	1
IF	A	71	4	1	1	2
IF	A	71	3	1	1	3
IF	A	71	4	1	1	3
IF	A	71	5	2	1	2
IF	C	71	4	1	1	1
IF	C	71	3	1	1	2
IF	C	71	2	1	1	1
IF	C	71	4	1	1	2
IF	C	71	5	1	1	1
IF	C	71	3	3	1	4
IF	B	71	3	1	1	2
IF	B	71	2	1	1	2
IF	B	71	4	1	1	3
IF	B	71	3	1	1	1
IF	B	71	4	1	1	2
IF	B	71	4	1	1	2
IF	B	71	4	3	3	1
IF	B	71	5	1	1	3
IF	B	71	3	1	1	3
IF	B	71	2	1	2	2
IF	B	71	1	1	1	4
IF	B	71	4	2	1	2
IF	B	71	3	2	3	2
IF	B	71	2	2	3	1
IF	B	71	5	2	4	4
IF	B	71	3	2	1	2
IF	B	71	4	1	1	1
IF	B	71	2	2	2	3
LD	B	71	2	1	1	1
LD	B	71	3	1	1	1
LD	B	71	3	1	1	1
LD	B	71	3	1	1	1
LD	B	71	3	1	1	1
LD	B	71	3	1	1	1
LD	B	71	4	1	1	1

LD	B	71	3	2	2	2
LD	B	71	5	1	1	1
LD	B	71	4	1	1	1
LD	B	71	3	1	1	1
LD	B	71	4	1	1	1
SERR	C	71	2	1	2	2
SERR	C	71	3	1	1	2
SERR	C	71	2	2	1	2
SERR	C	71	3	1	1	1
SERR	C	71	4	1	1	1
SERR	C	71	5	1	1	2
SERR	B	71	4	2	1	1
SERR	B	71	2	1	1	3
SERR	B	71	2	2	1	1
SERR	B	71	3	1	1	1
SERR	B	71	3	1	1	1
SERR	B	71	3	1	1	1
SERR	B	71	4	1	1	1
SERR	B	71	3	1	1	1
SERR	B	71	4	1	1	2
SERR	B	71	3	2	2	1
SERR	B	71	3	1	1	1
SERR	B	71	4	1	1	1
SS	C	71	5	1	1	2
SS	C	71	4	1	1	1
SS	C	71	3	1	1	1
SS	C	71	3	1	1	1
SS	C	71	3	1	1	2
SS	C	71	3	3	2	4
SS	B	71	3	1	1	3
SS	B	71	3	2	1	2
SS	B	71	3	1	1	1
SS	B	71	3	1	1	1
SS	B	71	5	1	1	1
SS	B	71	3	1	1	1
TM	A	71	4	1	1	1
TM	A	71	4	1	1	1
TM	A	71	4	1	1	1
TM	A	71	4	1	1	1
TM	A	71	4	1	1	1

TM	A	71	4	1	1	1
TM	B	71	3	1	1	1
TM	B	71	1	3	2	1
TM	B	71	4	4	1	1
TM	B	71	2	3	2	1
TM	B	71	4	1	2	1
TM	B	71	2	2	1	2
IF	C	82	3	3	3	5
IF	C	82	4	1	1	2
IF	C	82	4	1	1	1
IF	C	82	3	1	1	1
IF	C	82	2	2	1	2
IF	C	82	3	3	2	3
IF	B	82	5	1	1	3
IF	B	82	3	3	2	4
IF	B	82	2	2	2	1
IF	B	82	5	1	1	3
IF	B	82	3	1	1	2
IF	B	82	4	3	2	2
IF	B	82	2	3	3	1
IF	B	82	3	1	1	2
IF	B	82	3	1	1	1
IF	B	82	3	1	1	2
IF	B	82	4	1	1	1
IF	B	82	3	2	1	3
IF	B	82	3	1	1	1
IF	B	82	4	1	1	1
IF	B	82	5	1	2	3
IF	B	82	4	1	1	1
IF	B	82	5	1	1	2
IF	B	82	3	4	2	1
LD	B	82	3	1	1	1
LD	B	82	4	1	2	2
LD	B	82	4	1	1	1
LD	B	82	2	3	1	1
LD	B	82	5	1	1	1
LD	B	82	2	1	1	1
SERR	B	82	3	1	2	1
SERR	B	82	3	1	1	1
SERR	B	82	3	1	1	1

SERR	B	82	3	1	1	1
SERR	B	82	2	2	2	1
SERR	B	82	2	3	4	3
SERR	B	82	3	2	1	1
SERR	B	82	2	2	2	1
SERR	B	82	2	3	1	2
SERR	B	82	3	2	1	1
SERR	B	82	3	1	1	1
SERR	B	82	3	3	3	1
SS	A	82	3	3	1	4
SS	A	82	4	1	1	2
SS	A	82	4	1	1	2
SS	A	82	3	1	1	1
SS	A	82	2	1	1	1
SS	A	82	2	2	1	2
SS	A	82	3	1	1	1
SS	A	82	5	1	1	1
SS	A	82	3	1	1	1
SS	A	82	3	1	1	1
SS	A	82	5	2	1	2
SS	A	82	4	2	1	1
TM	B	82	3	3	3	1
TM	B	82	4	1	1	1
TM	B	82	3	1	1	1
TM	B	82	3	1	1	1
TM	B	82	3	1	1	1
TM	B	82	4	1	1	1

Sensory panel scores (5-point intensity scale) for *infraspinatus* muscle after packaging and antioxidants injection (cooking temperature 74˚C)

PKG	PLANT	BEEFY	RANCID	METALIC	LIVER
PVC	A	3	2	3	3
PVC	A	1	4	4	1
PVC	A	2	1	2	1
PVC	A	3	1	1	1
PVC	A	1	1	4	1
PVC	A	2	2	3	2
PVC	B	4	1	1	1
PVC	B	3	2	2	2
PVC	B	2	2	1	2
PVC	B	3	1	2	1
PVC	B	3	1	3	1
PVC	B	4	1	1	
PVCAN	A	2	1	2	2
PVCAN	A	3	2	3	1
PVCAN	A	2	1	1	2
PVCAN	A	2	1	1	1
PVCAN	A	2	1	3	1
PVCAN	A	4	1	1	2
PVCAN	B	4	1	1	1
PVCAN	B	2	3	3	1
PVCAN	B	3	1	1	2
PVCAN	B	3	1	1	1
PVCAN	B	3	1	2	1
PVCAN	B	2	1	4	1
O280%	A	4	2	1	2
O280%	A	4	1	1	1
O280%	A	4	1	1	1
O280%	A	4	1	1	1
O280%	A	1	2	3	1
O280%	A	2	2	2	1
O280%	A	2	1	2	4
O280%	A	3	1	1	1
O280%	A	1	1	4	1
O280%	A	3	1	1	1

O280%	A	3	1	1	1
O280%	A	3	2	1	3
O280%AN	A	2	2	3	1
O280%AN	A	2	2	3	1
O280%AN	A	3	1	1	1
O280%AN	A	4	1	1	1
O280%AN	A	1	1	3	1
O280%AN	A	1	2	2	1
O280%AN	A	4	1	2	1
O280%AN	A	3	1	3	1
O280%AN	A	1	1	5	1
O280%AN	A	1	1	5	1
O280%AN	A	2	1	2	2
O280%AN	A	3	1	1	1
O280%AN	B	3	2	4	2
O280%AN	B	3	2	2	1
O280%AN	B	4	1	1	1
O280%AN	B	3	1	1	1
O280%AN	B	1	1	5	2
O280%AN	B	2	1	2	1
O280%AN	B	2	1	3	1
O280%AN	B	1	1	1	4
O280%AN	B	1	1	4	1
O280%AN	B	2	1	2	1
O280%AN	B	3	1	2	1
O280%AN	B	3	1	2	1
COMAP	A	3	1	1	2
COMAP	A	2	1	1	3
COMAP	A	2	1	1	2
COMAP	A	3	1	1	1
COMAP	A	3	3	4	2
COMAP	A	3	3	4	3
COMAP	A	4	1	1	1
COMAP	A	5	1	1	1
COMAP	A	4	1	1	1
COMAP	A	3	1	1	1
COMAP	A	4	1	1	1
COMAP	A	3	1	1	2
COMAPAN	A	2	1	1	3

COMAPAN	A	3	1	1	3
COMAPAN	A	4	1	1	1
COMAPAN	A	3	1	1	1
COMAPAN	A	4	3	1	1
COMAPAN	A	4	3	2	1
COMAPAN	A	3	1	1	1
COMAPAN	A	3	1	1	1
COMAPAN	A	3	1	1	1
COMAPAN	A	3	1	1	1
COMAPAN	A	3	2	1	3
COMAPAN	A	3	2	1	2

Thiobarbituric acid values (TBA) for five select grade beef chuck muscles

MUSCLE	PLANT	TEMP (˚C)	TBA #*
IF	A	71	0.23
IF	B	71	0.16
IF	B	82	0.20
IF	B	71	0.20
IF	B	82	0.21
IF	B	71	0.34
IF	B	71	0.52
IF	B	82	0.54
IF	B	82	0.58
IF	A	71	0.39
IF	B	71	0.14
IF	B	71	0.19
IF	B	82	0.16
IF	B	82	0.14
IF	C	71	0.16
IF	C	82	0.18
LD	B	71	0.07
LD	B	71	0.21
LD	B	71	0.28
LD	B	82	0.25
LD	B	82	0.28
LD	A	71	0.23
SERR	B	71	0.20
SERR	B	82	0.34
SERR	B	71	0.18
SERR	B	82	0.23
SERR	B	71	0.23
SERR	B	71	0.31
SERR	B	82	0.28
SERR	B	82	0.26
SERR	A	71	0.45
SS	B	71	0.17
SS	B	82	2.49
SS	A	82	0.40
TM	A	71	0.14

TM	B	71	0.18
TM	A	71	0.78
TM	C	71	0.14
TM	C	82	0.22

*TBA # = Absorbance @ 535 nm x 2.77

TBA values for select grade *infraspinatus* muscle sample after packaging and antioxidant treatment (cooking temperature = 74˚C)

PKG	STORAGE (DAYS)	PLANT	COOKTEMP	TBA
PVC5D	5	A	74	1.16
PVC5D	5	A	74	1.2
PVC5D	5	B	74	0.45
PVC5D	5	B	74	0.47
PVC5DAN	5	A	74	0.63
PVC5DAN	5	A	74	0.53
PVC5DAN	5	B	74	0.55
PVC5DAN	5	B	74	0.6
O280%	10	A	74	1.61
O280%	10	A	74	1.63
O280%	10	B	74	0.73
O280%	10	B	74	0.74
O280%AN	10	B	74	3.31
O280%AN	10	B	74	2.78
COMAP	21	A	74	0.37
COMAP	21	A	74	0.32
COMAPAN	21	A	74	0.45
COMAPAN	21	A	74	0.48

Figure 10 HPLC standard curve for myoglobin

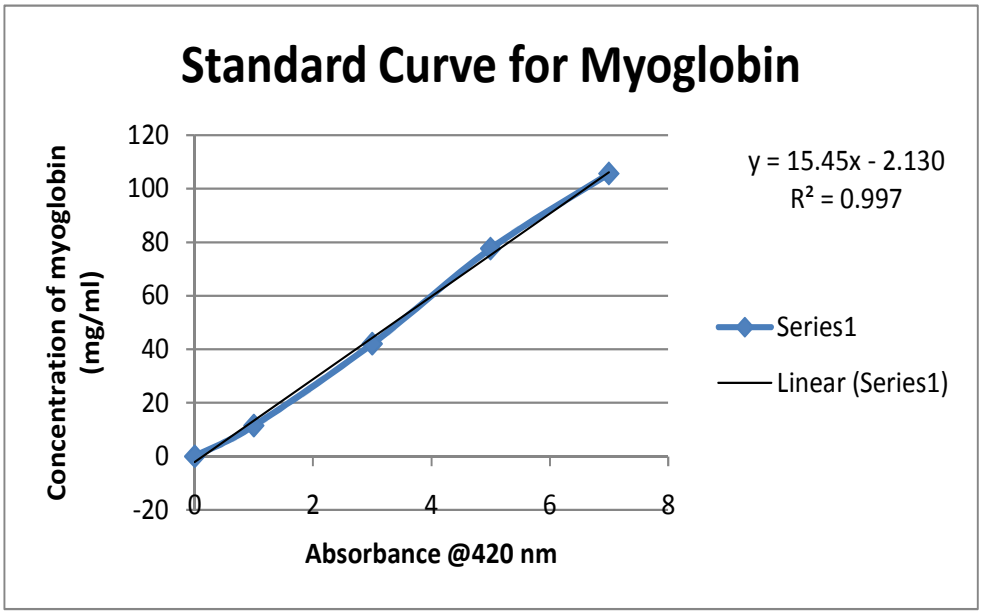
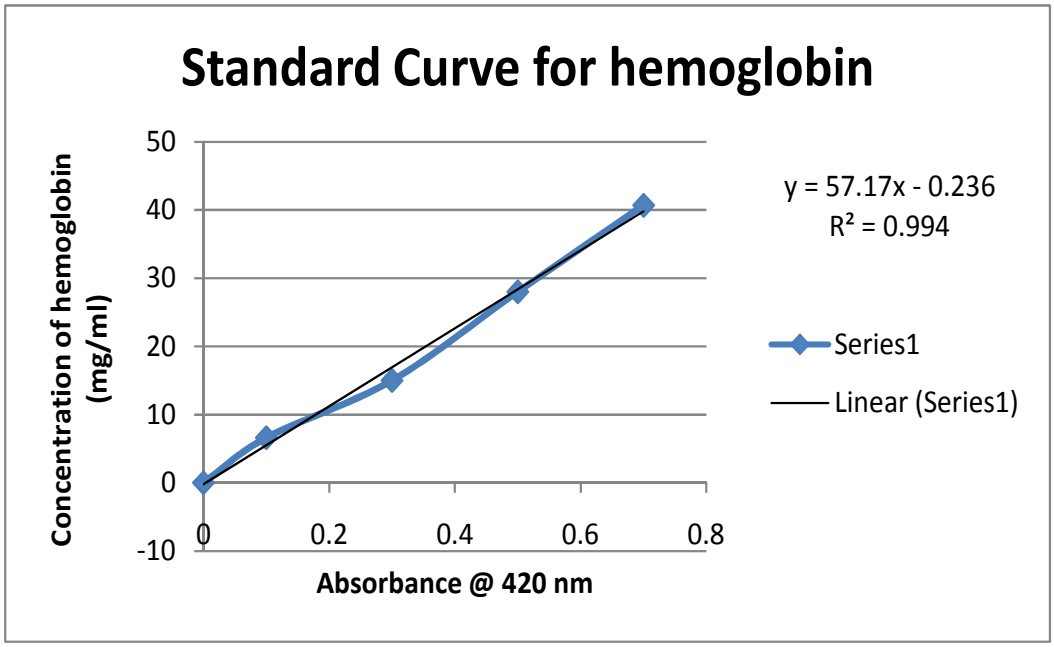


Figure 11 HPLC standard curve for hemoglobin



Statistics:

Statistica General Manova	**Means (± standard deviation)**			
Muscle	**L***	**a***	**b***	**N**
IF	35.06±1.29	9.67±3.34	14.08±2.47	5
TM	36.02±5.87	9.81±3.87	13.36±2.49	3
SERR	32.81±5.86	11.01±7.78	14.45±5.86	3
SS	33.31±3.27	8.88±5.54	13.69±4.24	3
LD	37.47±1.52	8.58±5.68	13.81±1.93	3
PVC	34.64±2.12	7.93±2.34	13.35±2.36	3
PVCAN	37.38±3.91	10.01±5.00	15.74±5.03	3
80%O2	39.72±3.03	7.65±1.98	13.7±2.18	2
80%O2AN	36.36±0.00	6.33±0.00	14.37±0.00	1
COMAP	34.72±4.21	20.19±2.90	14.22±0.47	2
COMAPAN	36.47±3.90	20.27±1.25	14.69±0.31	2

Statistica General Manova	Main Effect: Muscle			
Dependent Variable	Mean Sqr Effect	Mean Sqr Error	F (df1,2) 10, 19	p-level
L*	10.14	13.29	0.76	0.66
a*	45.69	20.89	2.19	0.06
b*	1.37	11.38	0.12	0.99

Statistica General Manova	**Means (± standard deviation)**	
Plant	**Total Mb**	**N**
B	5.06±1.17	16
A	5.21±1.17	8
C	7.12±0.08	2

Statistica General Manova	Main Effect: Plant				
Test	of Sqrs	df	Mean Sqr	F	p-level
Effect	7.59	2	3.79	2.87	0.07
Error	30.37	23	1.32		

Statistica General Manova	**Means (± standard deviation)**	
Muscle	**Total Mb**	**N**
TM	3.60±0.25	2
IF	5.42±1.27	16
SERR	5.71±1.49	4
SS	5.01±0.30	2
LD	4.97±0.16	2

Statistica General Manova	Main Effect: Muscle				
Univar. Test	Sum of Sqrs	df	Mean Sqr	F	p-level
Effect	7.07	4	1.76	1.2	0.34
Error	30.89	21	1.47		

APPENDIX B

DATA FOR CHAPTER V

Trout Procedure for Total pigment in the Infraspinatus muscle sample

Sample	Replicate	A525	A572	A700	Total Pigment (mg/mL)
Old Cow1	1	0.4112	0.5871	0.0002	9.042
	2	0.4158	0.5851	0	9.1476
Old Cow2	1	0.3446	0.3385	0	7.5812
	2	0.4098	0.2918	0	9.0156
	3	0.5951	0.8263	0	13.0922
	4	0.3591	0.2346	0.0005	7.8892
Old Cow3	1	0.2964	0.2804	0	6.5208
	2	0.3112	0.1722	0	6.8464

Hornsey B Procedure for Total Hematin content of Infraspinatus muscle samples

Sample	Replicate	A640	Total Hematin (ppm)
Old Cow1	1	0.3244	220.592
	2	0.2658	180.744
	3	0.3007	204.476
	4	0.3141	213.588
Old Cow2	1	0.2635	179.18
	2	0.3371	229.228
	3	0.3621	246.228
	4	0.373	253.64
Old Cow3	1	0.2974	202.232
	2	0.2967	201.756
	3	0.3504	238.272
	4	0.3015	205.02

Hunter Color Measurement for L*, a* and b* values (Old Cow Infraspinatus muscle)

Day	Packaging	Average Value			
		L*	a*	b*	Visual
0	No Packaging	26.88	8.61	10.26	Purple
0	No Packaging	30.47	9.47	11.11	Purple
0	No Packaging	39.97	5.72	6.36	Purple
5	PVC-wrap	25.92	9.27	11.82	Red/Brown
5	PVC-wrap	32.54	8.41	11.13	Red/Brown
5	PVC-wrap	35.02	8.52	13.01	Red/Brown
5	PVC-wrap (AO)	26.2	10.88	12.58	Red/Brown
5	PVC-wrap (AO)	32.7	11.14	12.21	Red/Brown
5	PVC-wrap (AO)	33.07	15.59	11.67	Red/Brown
10	80% O2-MAP	41.34	16.35	16.94	Red
10	80% O2-MAP	40.85	15.52	14.92	Red
10	80% O2-MAP	41.46	18.39	20.26	Red
10	80% O2-MAP (AO)	36.03	15.74	19.61	Red
10	80% O2-MAP (AO)	40.87	14.01	15.34	Red
10	80% O2-MAP (AO)	42.03	19.9	23.01	Red
21	0.4% CO-MAP	38.42	23.05	14.77	Bright Red
21	0.4% CO-MAP	42.25	19.77	14.25	Bright Red
21	0.4% CO-MAP	43.24	23.54	14.74	Bright Red
21	0.4% CO-MAP (AO)	44.27	19.7	13.27	Bright Red
21	0.4% CO-MAP (AO)	43.4	24.61	16.27	Bright Red
21	0.4% CO-MAP (AO)	39.16	20.21	15.27	Bright Red

Average sensory score for Old cow Infraspinatus muscle (different flavor and tenderness attribute) by trained panel

Sample	Beefiness	Metallic	Rancid	Liver	Sour/Grassy	Tender	Tough
Control	5	1	1	1	1	3	1
Control	1	1	1	2	3	3	1
Control	1	1	2	3	1	5	1
Control	3	1	1	1	4	3	1
Control	5	1	1	1	1	3	1
PVC wrap 1	1	1	1	1	4	1	2
PVC wrap 1	3	1	1	1	1	2	3
PVC wrap 1	3	1	3	1	3	1	3
PVC wrap 1	5	1	1	1	1	1	2
PVC wrap 1	5	1	1	1	1	3	1
PVC wrap 2	4	1	1	1	2	1	5
PVC wrap 2	2	1	1	1	2	2	4
PVC wrap 2	2	1	2	1	3	1	5
PVC wrap 2	4	1	1	1	1	1	2
PVC wrap 2	3	1	1	1	1	2	1
PVC wrap 3	1	1	1	1	4	1	5
PVC wrap 3	2	1	1	2	3	2	4
PVC wrap 3	5	1	1	1	1	4	1
PVC wrap 3	2	1	2	1	2	1	5
PVC wrap 3	4	1	1	1	1	1	3
PVC w/AO 1	4	1	1	1	2	4	1
PVC w/AO 1	2	1	1	1	2	4	1
PVC w/AO 1	1	1	2	2	1	4	1
PVC w/AO 1	2	1	2	1	3	1	2
PVC w/AO 1	1	1	1	1	1	5	1
PVC w/AO 2	4	1	1	1	4	3	1
PVC w/AO 2	2	1	1	1	1	2	3
PVC w/AO 2	2	1	1	3	1	4	1
PVC w/AO 2	1	1	1	1	1	1	3
PVC w/AO 2	1	1	1	1	1	5	1
PVC w/AO 3	4	1	1	1	1	2	1
PVC w/AO 3	1	1	1	1	1	2	2
PVC w/AO 3	1	1	1	1	2	5	1
PVC w/AO 3	3	1	1	1	1	1	2
PVC w/AO 3	1	1	1	1	2	4	1

Sample	Beefiness	Metallic	Rancid	Liver	Sour/Grassy	Tender	Tough
Control	1	1	1	1	1	1	1
Control	1	1	3	1	2	1	2
Control	2	1	4	1	2	1	3
Control	2	1	4	1	3	2	3
Control	3	1	5	3	5	2	4
80% O2-1	1	1	1	1	1	1	2
80% O2-1	2	1	2	1	1	1	3
80% O2-1	2	1	2	1	2	1	3
80% O2-1	2	1	3	1	3	2	4
80% O2-1	4	1	4	2	4	2	5
80% O2-2	1	1	1	1	1	1	1
80% O2-2	2	1	1	1	1	2	1
80% O2-2	3	1	1	1	1	2	3
80% O2-2	4	1	2	2	2	3	3
80% O2-2	4	1	3	2	3	3	4
80% O2-3	1	1	1	1	1	1	1
80% O2-3	1	1	1	1	1	1	1
80% O2-3	2	1	1	1	1	3	2
80% O2-3	2	1	2	2	1	4	3
80% O2-3	3	1	2	4	5	5	4
80% O2 (with AO)-1	1	1	3	1	1	1	1
80% O2 (with AO)-1	1	1	3	1	2	1	1
80% O2 (with AO)-1	2	1	3	1	2	2	3
80% O2 (with AO)-1	2	1	4	1	2	2	3
80% O2 (with AO)-1	2	1	4	1	3	2	5
80% O2 (with AO)-2	2	1	1	1	1	1	1
80% O2 (with AO)-2	2	1	1	1	1	1	2
80% O2 (with AO)-2	3	1	2	1	3	1	2
80% O2 (with AO)-2	3	1	2	1	3	2	4
80% O2 (with AO)-2	3	2	2	3	5	3	5
80% O2 (with AO)-3	2	1	1	1	1	3	1
80% O2 (with AO)-3	2	1	1	1	1	3	1
80% O2 (with AO)-3	3	1	1	1	2	3	1
80% O2 (with AO)-3	4	1	2	1	3	4	1
80% O2 (with AO)-3	4	2	3	2	3	4	1

Sample	Beefiness	Metallic	Rancid	Liver	Sour/Grassy	Tender	Tough
Control	2	2	1	1	2	2	3
Control	3	1	1	1	3	1	3
Control	4	1	1	1	3	1	2
Control	2	1	1	1	5	1	2
Control	3	1	3	3	3	2	3
CO-MAP-1	4	1	1	1	1	2	1
CO-MAP-1	4	1	1	2	1	2	4
CO-MAP-1	1	1	3	1	5	2	1
CO-MAP-1	2	1	1	1	1	2	1
CO-MAP-1	2	1	1	4	4	1	2
CO-MAP-2	2	1	1	1	1	3	1
CO-MAP-2	2	2	1	1	2	2	3
CO-MAP-2	1	1	5	1	4	1	4
CO-MAP-2	2	1	1	1	2	1	2
CO-MAP-2	2	1	1	1	3	4	1
CO-MAP-3	2	1	1	1	1	4	1
CO-MAP-3	2	1	3	1	3	1	3
CO-MAP-3	2	1	2	1	4	1	3
CO-MAP-3	2	1	1	1	1	3	1
CO-MAP-3	3	1	4	2	1	3	1
CO-MAP with AO-1	4	1	1	1	1	3	1
CO-MAP with AO-1	2	1	3	1	3	2	3
CO-MAP with AO-1	4	1	1	1	1	2	1
CO-MAP with AO-1	3	1	1	1	3	1	2
CO-MAP with AO-1	3	1	4	1	2	3	1
CO-MAP with AO-2	3	1	1	1	1	3	1
CO-MAP with AO-2	3	1	1	1	2	3	2
CO-MAP with AO-2	2	1	3	1	2	2	1
CO-MAP with AO-2	3	1	1	1	2	2	1
CO-MAP with AO-2	3	1	1	3	1	3	1
CO-MAP with AO-3	4	1	1	1	1	2	1
CO-MAP with AO-3	2	1	2	1	3	3	1
CO-MAP with AO-3	3	1	4	1	3	2	1
CO-MAP with AO-3	3	1	1	1	2	3	1
CO-MAP with AO-3	3	1	2	1	3	1	4

TBA Values for Old Cow Infraspinatus muscle sample after cooking at 74°C

Sample	Packaging	A532	TBA#
Control	No packaging	0.2125	0.588625
Control	No packaging	0.2265	0.627405
Old Cow1	PVC-wrap (No AO)	0.3218	0.891386
	PVC-wrap (No AO)	0.3326	0.921302
Old Cow2	PVC-wrap (No AO)	0.3986	1.104122
	PVC-wrap (No AO)	0.4064	1.125728
Old Cow3	PVC-wrap (No AO)	0.3818	1.057586
	PVC-wrap (No AO)	0.3645	1.009665
Old Cow1	PVC-wrap (With AO)	0.2359	0.653443
	PVC-wrap (With AO)	0.2063	0.571451
Old Cow2	PVC-wrap (With AO)	0.1925	0.533225
	PVC-wrap (With AO)	0.2166	0.599982
Old Cow3	PVC-wrap (With AO)	0.3087	0.855099
	PVC-wrap (With AO)	0.3159	0.875043

Sample	Packaging	A532	TBA#
Control	No packaging	0.542	1.50134
Control	No packaging	0.498	1.37946
Old Cow1	80% O2-MAP (No AO)	0.148	0.40996
	80% O2-MAP (No AO)	0.135	0.37395
Old Cow2	80% O2-MAP (No AO)	0.145	0.40165
	80% O2-MAP (No AO)	0.121	0.33517
Old Cow3	80% O2-MAP (No AO)	0.168	0.46536
	80% O2-MAP (No AO)	0.165	0.45705
Old Cow1	80% O2-MAP (With AO)	0.085	0.23545
	80% O2-MAP (With AO)	0.078	0.21606
Old Cow2	80% O2-MAP (With AO)	0.076	0.21052
	80% O2-MAP (With AO)	0.071	0.19667
Old Cow3	80% O2-MAP (With AO)	0.082	0.22714
	80% O2-MAP (With AO)	0.075	0.20775

Sample	Packaging	A532	TBA#
Control	No packaging	0.1196	0.331292
Control	No packaging	0.147	0.40719
Old Cow1	0.4% CO-MAP (No AO)	0.1141	0.316057
	0.4% CO-MAP (No AO)	0.0925	0.256225
Old Cow2	0.4% CO-MAP (No AO)	0.1637	0.453449
	0.4% CO-MAP (No AO)	0.1346	0.372842
Old Cow3	0.4% CO-MAP (No AO)	0.1709	0.473393
	0.4% CO-MAP (No AO)	0.2224	0.616048
Old Cow1	0.4% CO-MAP (With AO)	0.1576	0.436552
	0.4% CO-MAP (With AO)	0.2008	0.556216
Old Cow2	0.4% CO-MAP (With AO)	0.1786	0.494722
	0.4% CO-MAP (With AO)	0.2086	0.577822
Old Cow3	0.4% CO-MAP (With AO)	0.1914	0.530178
	0.4% CO-MAP (With AO)	0.1761	0.487797

Warner Brazler Shear Force Data

Sample	Reading (Kg)	Sample	Reading (Kg)	Sample	Reading (Kg)
PVC wrap1	1.1	80% O2-1	2.6	CO-MAP-1	3.4
PVC wrap1	2.3	80% O2-1	2.5	CO-MAP-1	2.9
PVC wrap1	1.7	80% O2-1	1.6	CO-MAP-1	2.5
PVC wrap2	3.1	80% O2-2	2.2	CO-MAP-2	1.2
PVC wrap2	3.8	80% O2-2	3.7	CO-MAP-2	1.4
PVC wrap2	5.6	80% O2-2	2	CO-MAP-2	1.4
PVC wrap3	3	80% O2-3	2.5	CO-MAP-3	3.6
PVC wrap3	4	80% O2-3	2.5	CO-MAP-3	3
PVC wrap3	3.8	80% O2-3	3.2	CO-MAP-3	2.2
PVC w/AO 1	3.5	80% O2 with AO-1	1.5	CO-MAP with AO-1	3.5
PVC w/AO 1	1.1	80% O2 with AO-1	2.5	CO-MAP with AO-1	2.5
PVC w/AO 1	1.7	80% O2 with AO-1	2.5	CO-MAP with AO-1	3.1
PVC w/AO 2	1.2	80% O2 with AO-2	2.1	CO-MAP with AO-2	2.9
PVC w/AO 2	2.4	80% O2 with AO-2	2.2	CO-MAP with AO-2	3.7
PVC w/AO 2	3	80% O2 with AO-2	2.6	CO-MAP with AO-2	4.3
PVC w/AO 3	1.7	80% O2 with AO-3	1.7	CO-MAP with AO-3	3.6
PVC w/AO 3	3.5	80% O2 with AO-3	1.9	CO-MAP with AO-3	1.9
PVC w/AO 3	2.1	80% O2 with AO-3	1.8	CO-MAP with AO-3	3.4

Statistics:

Raw *infraspinatus* (Commercial and Select grade) muscle

Statistica General Manova	Main effect: Treatment			
Dependent Variable	Mean Sqr. Effect	Mean Sqr. Error	f (df 1,2) 1, 10	p-level
Phosphate extractable pigment	31.04	3.97	7.81	0.02
Hornsey acetone extractable hematin	31503.38	801.95	39.28	0.00
L*	16.54	18.53	0.89	0.37
a*	22.54	2.61	8.61	0.01
b*	76.91	4.79	16.04	0.00
Hue angle (atan (b*/a*)	0.02	0.003	5.97	0.03

Treated and cooked *infraspinatus* (Commercial and Select grade) muscle

tatistica General Manov	Main effect: Treatment (packaging and antioxidants)			
Dependent Variable	Mean Square Effect	Mean Square Error	f (df 1,2) 1, 10	p-val
Beefiness	2.92	1.093	2.674	0.03
Metallic	0.046	0.031	1.47	0.21
Rancid	2.346	0.931	2.518	0.04
Livery	0.453	0.334	1.353	0.25
Sour/grassy	0.864	1.317	0.656	0.66
Tenderness	3.717	1.244	2.987	0.02
Toughness	6.251	1.504	4.154	0.00

Table of all effects (MANCOVA)

Effect	df Effect	Mean Square Effect	df Error	MS Error	F	p-level
WBS	5	2.03	48	0.76	2.63	0.03
TBA	5	0.46	30	0.01	52.72	0.00

APPENDIX C

PHOTOGRAPHS OF INFRASPINATUS MUSCLE IN DIFFERENT PACKAGING ATMOSPHERE (WITH AND WITHOUT ANTIOXIDANT INJECTION

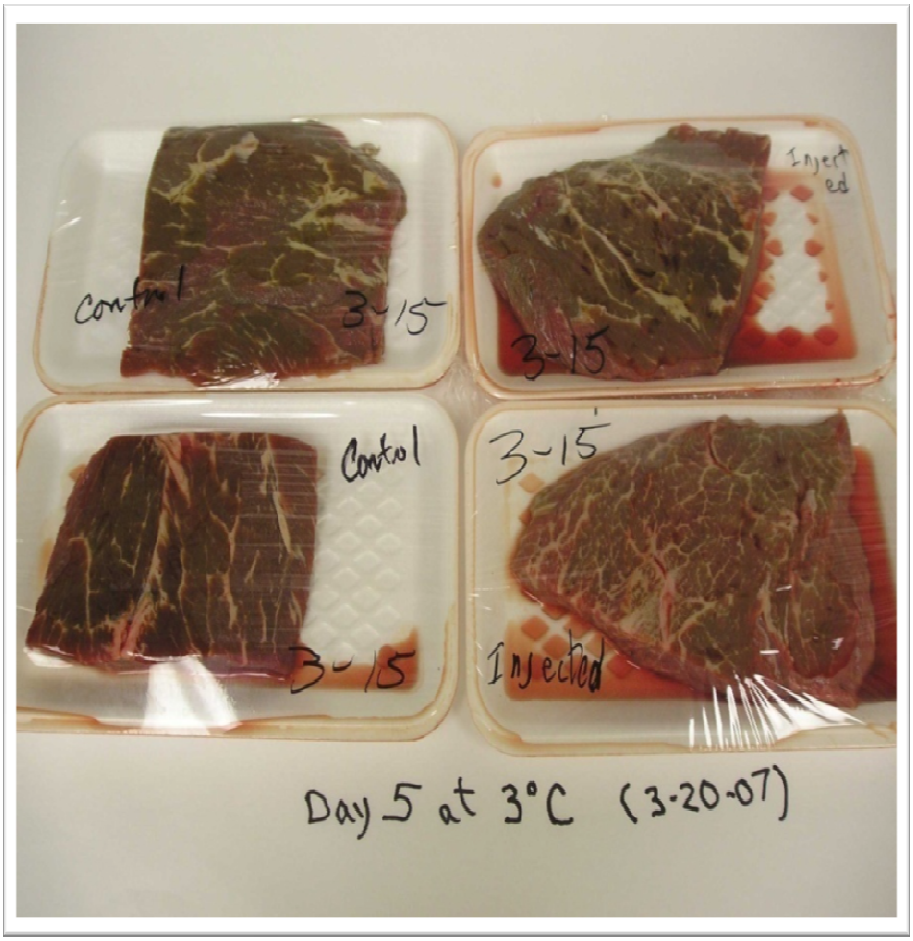


Photo 1. *Infraspinatus* in PVC-overwrap [w/o antioxidant injection (left) with antioxidant injection (right)] after 5 d @ 3°C.

Photo 2. *Infraspinatus* 80% O_2-MAP [w/o antioxidant injection (left) with antioxidant injection (right)] after 10 d @ 2˚C.

Photo 3. *Infraspinatus* in 0.4% CO-MAP [w/o antioxidant injection (left) with antioxidant injection (right)] after 21 d @ 2˚C.

APPENDIX D

ROTATION PLAN FROM SIMS 2000 SOFTWARE FOR DESCRIPTIVE PANEL

ROTATION PLAN FOR SIMS 2000 SENSORY SOFTWARE

Test Definition Code.....: T7-PANEL

Test Definition Description: RanjeetaM

Experimental Design: T7-PANEL Experimental Plan: 04040001

Design Type: Descriptive Number of Samples: 4

Number of Reps: 1 Number Presented: 4

Number of Panelists: 6 Number of Blocks: 1

Screen Sequencing: Uni-Directional Number of Reps: 1

Block Order: As in Plan

Samples Order: As in Plan

--> NOT PERFECTLY BALANCED <--

	Pos 1	Pos 2	Pos 3	Pos 4	Totals
Sample 1	6	0	0	0	6
Sample 2	0	6	0	0	6
Sample 3	0	0	6	0	6
Sample 4	0	0	0	6	6

Block Assignment Analysis Not Included

Sample Descriptions:

Sample 1 Sample 1 – Control sample cooked at 74C (527)

Sample 2 Sample 2 – 80% Oxygen-modified atmosphere packaged and injected with antioxidants, cooked at 74C after 10 days of storage (364)

Sample 3 Sample 3 – Duplicate sample of 364 (471)

Sample 4 Sample 4 – Duplicate sample of 527 (875)

ROTATION PLAN: BY SAMPLE SET

Experimental Definition: T7-PANEL

Rep: 1

Sample

Set	PanelistID	Panelist Name	Sample Order (Sample#/Sample Code)
1	0000000001	Resp # 1	1-527 2-364 3-875 4-471
2	0000000002	Resp # 2	1-364 2-527 3-471 4-875
3	0000000003	Resp # 3	1-471 2-527 3-875 4-364
4	0000000004	Resp # 4	1-364 2-471 3-527 4-875
5	0000000005	Resp # 5	1-471 2-875 3-364 4-527
6	0000000006	Resp # 6	1-875 2-364 3-527 4-471